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IMMOBILIZED ENZYMES/BACTERIA FOR NAVAL APPLICATIONS

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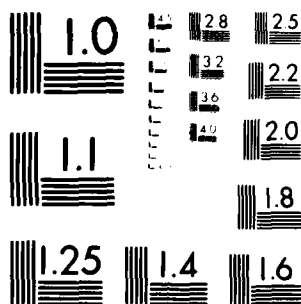
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OFFICE OF NAVAL RESEARCH
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Task No. NR 205-041
FINAL REPORT

Immobilized Enzymes/Bacteria for
Naval Applications - Initial Data Base

by

E. Findl
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J. Johnsen

BioResearch, Inc.
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May 1981

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Biotechnology and particularly enzyme technology, is a rapidly growing, high-potential area for research and development. In particular, immobilization of biocatalysts had been found useful. To date, this technology has been applied in the food, pharmaceutical, and related industries. However, naval applications can also be envisioned, despite the environmental difficulties present in military scenarios. Evaluation of enzyme and biotechnology for naval applications is therefore required. This report describes		

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the first phase of a program to survey potential Naval applications of immobilized biocatalysts. This report includes a general classification of the literature, an initial survey, a report on computerization of the data base, and specific reports on possible Naval applications.

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CONTRACTOR CERTIFIES THAT PROMPT IDENTIFICATION AND TIMELY DISCLOSURE OF SUBJECT INVENTIONS PROCEDURES HAVE BEEN FOLLOWED		
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1.0 Introduction

Biotechnology is now widely recognized as a scientific field with extremely high growth potential, as evidenced by the wide interest shown in "genetic engineering". This, however, is only one facet of the field. A more immediately applicable segment of the field is the use of enzymes. Enzymes are already being used in tasks ranging from industrial chemical production to clinical chemistry. All these tasks can be made more cost-effective by the development of more active and/or more stable enzymes. In most cases, this development is expected to entail the immobilization of the enzymes.

Immobilized enzymes have potential military applications as well as industrial uses. Among these potential applications are pollution control, air and water purification and detoxification, sensors for CBW applications, energy production, and a variety of medical uses. However, military applications can present difficulties not seen in industrial situations. Military applications tend to require serviceability under harsh, less well-controlled conditions, by less technically qualified personnel, perhaps under emergency conditions requiring fail-safe operation. Thus, although the potential is clearly present, evaluation of biotechnology and enzyme technology for the Navy, or for the military as a whole, requires special allowances for these special problems.

This report describes the first phase of a program to survey potential Naval applications of immobilized enzymes. Included in this phase of the project are a general classification of the literature, initiation of the detailed survey, a report on the possible computerization of the survey, and specific reports on possible Naval applications based on on-site discussions with Naval personnel and extrapolations of potential enzyme-based processes described in the literature.

2.0 Technical Discussions

Following segments of this report will provide summaries of our efforts during the first phase of this project. Detailed results of the initial data survey will be found in section 4.0.

2.1 Data Classification

The wide range of enzymes available and the proliferation of variant names for the enzymes resulted in the establishment, in the late 50's, of an International Commission on Enzymes as part of the International Union of Biochemistry. The Commission has now published four compilations, the latest of which, in 1978, covers 2122 enzymes. The enzymes are divided into six general classes: oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases. Each class is subdivided into subclasses based on the molecule(s) acted upon, the chemical bond broken or formed, or the cofactor used in the reaction. Examples of this system for the oxidoreductase class are shown in Table 1. Separate classifications were established in this survey for immobilizations of coenzymes or cofactors, for immobilizations of whole cells or organelles, and for theoretical studies of biotechnology problems.

This classification system was chosen as the primary organizational reference. Thus, the tables attached are listed in their sequence by enzyme number according to the IUB recommendations. All immobilizations located for a given enzyme may be found under its corresponding number. Section 4.1 contains a list of all enzymes included in the data base to date.

At an early stage of the compilation, it was found advisable to include other organizational data with the abstracts of each item in the data base. Immobilization methods were categorized as outlined by Zaborsky (1973) and numbered as shown in Table 2. The immediate purpose of the work described in the item abstracted was also categorized and numbered as shown in Table 3. These additional data will facilitate later computerization of the data base, should this be desirable, and also are of aid in storage and retrieval of specific items.

The abstracts presented in section 4.1 are organized in tabular form. The IUB number, formal name, and common name(s) and reaction(s) catalyzed are shown for each enzyme. Then, for each citation in which the enzyme was employed, the method of immobilization, major results and any comments are listed. The appropriate citation, from the list in section 4.2, is identified in the column at the far right.

TABLE 1

Classification and Nomenclature of Enzymes

Classification Number	Classification	Number and Trivial Name of Example
1.0	Oxidoreductases	
1.1	Alcohol donor	1.1.1.1 Alcohol dehydrogenase
1.2	Carbonyl donor	1.2.1.9 Glyceraldehyde phosphate dehydrogenase
1.3	CH-CH donor	1.3.1.3 Cortisone reductase
1.4	CH-NH ₂ donor	1.4.3.4 Monoamine oxidase
1.5	C-NH donor	1.5.1.2 Pyrroline-S-carboxylate reductase
1.6	NADH donor	1.6.2.2 Cytochrome b ₅ reductase
1.7	Other nitrogenous compounds as donors	1.7.99.2 Nitric oxide reductase
1.8	Sulfur groups as donors	1.8.1.2 Sulfite reductase
1.9	Heme groups as hydrogen donors	1.9.3.1 Cytochrome oxidase (formerly called cytochrome a ₃)
1.10	Diphenols as hydrogen donors	1.10.3.1 o-Diphenol oxidase
1.11	H ₂ O ₂ as hydrogen acceptor	1.11.1.6 Catalase 1.11.1.7 Peroxidase
1.12	H ₂ as reductant	1.12.1.1 Hydrogenase
1.13	O ₂ as oxidant of single substrate	1.13.1.1 Catechol 1,2 oxygenase
1.14	O ₂ as oxidant of paired substrate	1.14.1.6 Steroid 11-β-hydroxylase

TABLE 2

Classification of Immobilization Methods

1. Covalent attachment to polymer
2. Intermolecular crosslinking
3. Copolymerization of enzyme and polymer
4. Physical adsorption
5. Entrapment within polymer matrix
6. Microencapsulation
7. Containment via semipermeable membranes
0. Miscellaneous

TABLE 3

Classification of Level of Advancement of Research

1. Theoretical analyses
2. Study of immobilization technique
3. Study of enzyme properties
4. Laboratory scale (research) system
5. Pilot-plant scale (development) system
6. Commercial operation
0. None of the above

TABLE 4

Classification of Purpose of Research

1. Enzymology - General
 - 1.1 Immobilized enzymes
 - 1.1.1 General
 - 1.1.1.1 General review articles
 - 1.1.1.2 Data compilations
 - 1.1.1.3 Lists of references
 - 1.1.1.4 Studies of immobilization methods
 - 1.1.1.5 Studies of immobilized enzymes properties
 - 1.1.1.6 Transport phenomena in immobilized enzymes
 - 1.1.1.7 Engineering of immobilized enzyme systems
 - 1.1.1.8 Concepts suitable for future use of immobilized enzymes
 - 1.1.1.9 Cofactor immobilization and processing
 - 1.1.2 Enzyme electrodes - General
 - 1.1.2.1 Medical applications
 - 1.1.2.2 Environmental applications
 - 1.1.2.3 Industrial applications
 - 1.1.2.4 Thermal enzyme probe
 - 1.1.2.5 Automated analyses

- 1.1.3 Food processing
 - 1.1.3.1 Glucose isomerization
 - 1.1.3.2 Lactose hydrolysis
 - 1.1.3.3 Polysaccharide reduction
 - 1.1.3.4 Brewing and fermentation
 - 1.1.3.5 Cheese processing
 - 1.1.3.6 Milk processing
- 1.1.4 Energy production
 - 1.1.4.1 Hydrogen production
 - 1.1.4.2 Fuel cells
- 1.1.5 Industrial waste treatment
 - 1.1.5.1 Removal of toxins
 - 1.1.5.2 Anti-fouling treatment
- 1.1.6 Medical and biochemical applications
 - 1.1.6.1 Detoxification of air and water
 - 1.1.6.2 Dialysis of body fluids
 - 1.1.6.3 O₂ and CO₂ transport
 - 1.1.6.4 Biopolymers sequencing
 - 1.1.6.5 Reaction mechanism studies
 - 1.1.6.6 Enzyme therapy
 - 1.1.6.7 Dental applications
 - 1.1.6.8 Blood antigen control
 - 1.1.6.9 Purification of biochemicals
 - 1.1.6.10 Artificial organs using immobilized enzymes
- 1.1.7 Pharmaceuticals
 - 1.1.7.1 Amino acid production
 - 1.1.7.2 Steroid derivatives
 - 1.1.7.3 Penicillin derivatives
- 1.1.8 General chemical manufacture
- 1.2 Immobilized cells
 - 1.2.1 General
 - 1.2.1.1 General review articles
 - 1.2.1.2 Data compilations
 - 1.2.1.3 Lists of references
 - 1.2.1.4 Studies of immobilization methods
 - 1.2.1.5 Studies of immobilized cells properties
 - 1.2.1.6 Transport phenomena in immobilized cell systems
 - 1.2.1.7 Engineering of immobilized cell systems
 - 1.2.1.8 Concepts suitable for future use of immobilized cells
 - 1.2.2 Immobilized cell electrodes - general
 - 1.2.2.1 Medical applications
 - 1.2.2.2 Environmental
 - 1.2.2.3 Industrial
 - 1.2.2.4 Thermal cell probe
 - 1.2.3 Food processing
 - 1.2.3.1 Glucose isomerization
 - 1.2.3.2 Lactose hydrolysis
 - 1.2.3.3 Polysaccharide reduction
 - 1.2.3.4 Brewing and fermentation
 - 1.2.3.5 Cheese processing
 - 1.2.3.6 Milk processing

- 1.2.4 Energy production
 - 1.2.4.1 Hydrogen production
 - 1.2.4.2 Fuel cells
- 1.2.5 Industrial waste treatment
 - 1.2.5.1 Removal of toxins
 - 1.2.5.2 Anti-fouling treatment
- 1.2.6 Medical applications
 - 1.2.6.1 Detoxification of air and water
 - 1.2.6.2 Dialysis of body fluids
 - 1.2.6.3 O₂ and CO₂ transport
 - 1.2.6.4 Biopolymers sequencing
 - 1.2.6.5 Reaction mechanism studies
 - 1.2.6.6 Immobilized cell therapy
 - 1.2.6.7 Dental applications
 - 1.2.6.8 Blood antigen control
 - 1.2.6.9 Artificial organs using immobilized cells
- 1.2.7 Pharmaceuticals
 - 1.2.7.1 Amino acid production
 - 1.2.7.2 Steroid derivatives
 - 1.2.7.3 Penicillin derivatives
- 1.2.8 General chemical manufacture

2.2 Specific Applications of Naval Interest

In the course of this survey, several studies have been located which present applications of immobilized biocatalysts with immediate or long-range potential for use in naval or military systems. This section of this report will highlight these studies, which may be divided into the following general areas: air and water pollution detection and purification (including CBW activities, and waste disposal), energy production, medical applications, and a miscellaneous category. In each area, efforts to date are of two types. In the more advanced systems, an immobilized-biocatalyst process is in the laboratory research or early development phase, with clear possibilities for Naval or military use. Other possibilities, in which the immobilization of the biocatalyst has not yet been accomplished but would clearly be of use in the proposed task, will also be examined. The discussions here will include briefly all relevant references acquired to date. If the reference has been entered into the data base, the appropriate reference number and enzyme number will be given. Unabstracted references mentioned in this discussion are listed at the end of the main body of the report.

2.2.1 Air and Water Monitoring and Treatment Systems

Applications of immobilized biocatalysts to these systems can be divided into subcategories dependent on whether the system is used for monitoring or for treatment and on whether the contaminant of interest is a pollutant or a toxin (chemical or biological in nature). Investigations into the use of biocatalysts in all these possible combinations have been initiated.

Methods for the detection of environmental contaminants appear to be predominantly electrochemical in nature. A series of devices (40, 131, 132, 133; see also Hoover, 1972; Goodson and Jacobs, 1976) have been developed for the electrochemical detection of cholinesterase (3.1.1.7) inhibitors. Inhibition of this enzyme, involved in neuromuscular transmission, is the primary mode of action of many CBW agents used as anti-personnel weapons and also of many pesticides employed in civilian and military environments. In all of these detectors, cholinesterase is immobilized in some form. An acylcholine substrate flows past or through the enzyme and, in the absence of inhibitor, is hydrolyzed to an electroactive species whose concentration is measured electrochemically. Introduction of inhibitor to the enzyme environment (by a variety of methods depending on whether the sample is gaseous or liquid and the expected type and concentration of inhibitor) causes a decreased enzyme activity and a resultant decrease in the concentration of electroactive product. This decrease is monitored and activates alarms and/or diagnostic equipment.

The general use of cholinesterase inhibitors in CBW agents clearly indicates the applicability of this system to military requirements. However, continued development is necessary to attain the reliability, operability, and durability needed in an active military environment. Similar devices could easily be designed to detect the presence of CBW agents operating on other metabolic systems.

Electrochemical detection is also the primary mode of operation of other systems using immobilized biocatalysts to detect and quantify pollutant levels. Karube et al. (125) have developed microbial electrode BOD sensors. One sensor detects the decrease in oxygen due to consumption by a layer of immobilized microbes, while the other is a fuel cell, measuring current produced by the metabolic activity of immobilized microbes in contact with wastewater. The first method (oxygen decrease detection) appears to be more generally applicable to contaminant detection problems. It may be applied to the detection of any specific contaminant desired by employing a biocatalyst which has a specific affinity for that contaminant and which utilizes oxygen in its metabolism of the contaminant. Thus, Neujahr and Kjellen (1980) have immobilized phenolhydroxylase to construct a phenol electrode. Hikuma et al. (1979) have immobilized whole bacterial cells to determine methanol, ethanol, and, in another publication (Hikuma et al. 1980), ammonia. Aizawa et al. (1974) have constructed a hydrogen peroxide sensor using immobilized catalase. Kobos et al. (1979) have used a different concept to build a nitrate-sensitive electrode. In this method, immobilized bacteria reduce nitrate to ammonia which can be detected by standard ammonia-sensing electrodes; however, these electrodes are subject to more interference from related substances.

The probes utilizing oxygen sensors are less subject to this type of interference; however, any other substance present in the sample which is metabolized and uses up oxygen will create problems. This problem can be minimized by using specific enzymes rather than whole cells. In many cases, however, a system of several enzymes and/or coenzymes is required for the desired metabolism to occur, thus making enzyme immobilization more difficult. In other cases, removal of the enzyme from the cellular environment destabilizes it so that immobilization of the enzyme is extremely inefficient. Isolation of the enzyme can also be a problem in many cases. These considerations apply equally well, of course, to immobilizations performed for other purposes.

Use of immobilized biocatalysts in the removal of contaminants is also an important research topic. Humphrey and Pye (1972) have fabricated a pilot system for removal of phenol from water using immobilized polyphenol oxidase. Klein et al. (1979) have accomplished the same objective using immobilized whole cells (*Candida tropicalis*). However, their study was aimed at elucidating the kinetics of the reaction and did not examine the removal of small amounts of phenol. Johnston (1976) has examined

the removal of sucrose from waste water using immobilized invertase. Smiley et al (45) have examined the use of immobilized amylase (3.2.1.1) to treat the starchy effluent from paper mills.

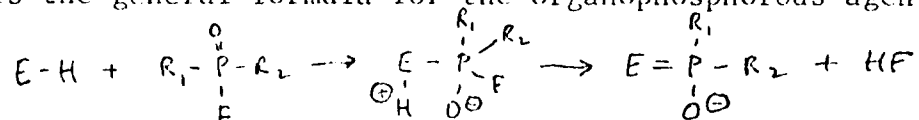
Several other systems for contaminant removal using biocatalysts have appeared in the literature which are more easily applicable to military or Naval needs. Cholson and Guire (1973) report the initial research on a system to modify enzymes to adhere to hydrocarbon-water interfaces. This could then be used, in principle, with hydrocarbon-oxidizing enzymes in treatment of oil slicks. However, hydrocarbon-oxidizing enzymes typically require cofactors; therefore, whole cell preparations are more likely to succeed. Hollo et al (1979) report the removal of nitrates and dissolved plutonium (among other heavy metals) by a packed bed of *Pseudomonas aeruginosa*. Up to 96% of dissolved Pu was removed, while the Pu concentrated in the packing could be more easily handled. Hancher et al (106) have developed a pilot plant level system for denitrification of nitrate-containing wastewaters from, among other sources, nuclear fuel reprocessing plants. The system uses whole bacterial cells which self-absorb on fine coal particles. The authors expect that the pilot plant can reduce 16 liters/min of $4,000 \text{ g NO}_3^-/\text{m}^3$ to $5 \text{ g NO}_3^-/\text{m}^3$.

Other research efforts have been aimed at the removal of pathogens and toxins using immobilized biocatalysts. Gainer et al (21) and Enright (1973) report investigations of the use of columns of enzymes immobilized on ceramic supports to destroy viruses in aerosol form passed through the column. Decreases of 2 logs (100 times) and more were found using immobilized ribonuclease (3.1.26.1) on strains of influenza, herpes, and Coxsackie viruses. Similar techniques were found effective for bacterial aerosols on *E. coli* and micrococcus strains. Henry (35) studied the use of immobilized peroxidase (1.11.1.7) on *E. coli* and *S. aureus*. Up to 85% of the cells were killed in a batch test; however, no continuous tests were made and there appeared to be a large variation depending on the source of the peroxidase used.

In the field of toxin removal by immobilized biocatalysts, Munnecke (43, 129, 130) has reported a series of studies based on the isolation from a mixed bacterial culture of an enzyme capable of hydrolyzing parathion (0,0-diethyl-0-nitrophenyl phosphorothioate), a commonly used insecticide. The enzyme was isolated and bound to porous glass and porous silica beads which were employed in a fluidized-bed reactor. At total flow rates of industrial wastewater up to 96 liter/hr and parathion concentrations up to 250 ppm, the system hydrolyzed 95% or more of the input parathion. Other pesticides, including 2,4-D, DDT, and aldrin are thought to be susceptible to such degradation (Munnecke, 1976). Since the organophosphate insecticides are closely related to several CBW agents (e.g., GB), this technique might prove adaptable to use in a detoxification system.

Another potentially useful concept in the CBW field involves the development of a direct indication of whether a specific localized area had actually been exposed and would therefore require decontamination. Such an indication would be sensitive to one or more of the agents likely to be employed at the expected dose level and should provide an irreversible, sharp indication of exposure.

Upon exposure to several organophosphate agents (including GB and Soman), the following reactions occur (where E - H is the active site of the cholinesterase molecule and $R_1 - \overset{\overset{O}{\parallel}}{P} - R_2$ is the general formula for the organophosphorous agents):



In the case of the most potent CBW agents, the last reaction is essentially irreversible, thus blocking the active site of the enzyme. Therefore, the detection of the presence of the CBW agent is equivalent to the detection of the product, HF. In the case of other agents, a similar reaction occurs, but the product released is different, including HCN and p-nitrophenol.

Detection of the small quantities of HF produced by lethal amounts of CBW agents can be accomplished in several ways. For example, a chemical can be included in the detector in a micro-encapsulated form which can be dissolved by HF. On dissolution, this chemical would react with a second chemical to give a colored species by an irreversible reaction. If the capsules are highly sensitive to HF, a visible reaction would result from contact with small amounts of the toxic agent.

Standard colorimetric methods for the detection of fluoride ion in water are effective down to 0.05 mg/l (50 parts per billion). The alizarin visual method appears very attractive, since a color comparator could be made up and either included on the badge or issued to personnel to be used with all the badges in a particular area. The precision and accuracy of this test are not as high as might be desired, but since the primary purpose of the test is as a "yes/no" indicator of contamination, quantitative accuracy is not critical.

2.2.2 Energy Production

Increased dependence on imported oil over the past decade has encouraged development of energy sources previously considered uneconomic. Among these sources are a whole spectrum of biologically produced substances which can be combusted or otherwise treated to produce energy. The biological origin of these substances naturally suggests the possibility of using biocatalysts to perform any necessary transformations.

Much of the energy-related biocatalyst research has been focused on the hydrolysis of starches and cellulosic materials produced by plants to the constituent simple sugars, primarily glucose, which can then be fermented or otherwise broken down to ethanol for use as a fuel feedback. Components of several such processes have been designed which employ immobilized enzymes, but physical size limitations imposed by military requirements would appear to limit their military application except insofar as they affect the overall U.S. energy situation.

It may be possible to utilize biocatalyzed processes to generate smaller amounts of energy synergistically while operating other processes. For example, Suzuki et al (104) and Karube et al (135) report development of a bio-fuel cell using immobilized whole cells of *Clostridium butyricum*. The fuel cell can be operated with a glucose feed or using industrial wastewater. The second application suggests that this system may provide an auxiliary power source that would be a "free" byproduct of a process that might be necessary in a closed environment (e.g. undersea or outer space).

Approaches to solar energy conversion using immobilized biocatalysis have been made by Egan and Scott (103) and by Hatchikian and Monsan (1980). These workers have immobilized hydrogenase enzymes and propose to use them along with immobilized chloroplasts and circulating ferredoxin (a coenzyme) to perform photosynthetic water-splitting. The reduced ferredoxin would be oxidized by the hydrogenase, producing hydrogen, and then re-reduced by the chloroplasts, using solar energy. Technical problems confronting this process are severe, however. A study by Wingard and Curecka (100) attempts a short cut. By immobilizing the cofactor to an electrode, they hoped to generate electrical energy directly from the reduction of the cofactor, in this case riboflavin. This work is still at too early a stage to predict a likely outcome.

2.2.3 Medical Applications

Because of their origin, one of the primary fields of application of immobilized biocatalysts has been the medical area. Two general areas of medical application may be identified, clinical analysis of biological samples and biocatalyst therapy. Concepts with general medical applicability and with specific military usefulness will be listed herein.

Clinical analyses for a great range of substances have been devised using immobilized enzymes. A partial list of quantifiable substances includes: glucose, glutamine, arginine, uric acid, urea, oxalate, phosphate, amino acids, arginase, methionine, cholesterol, alcohols, amygdalin (laetrile), tyrosine, antidiuretic hormone, ethanol, lactate, glycerol, choline, histidine, malate, penicillin, and triglycerides. The analyses are performed using immobilized enzymes to react with the substance and detecting the products with a variety of instruments, primarily oxygen electrodes, pH electrodes, and spectrophotometers. It appears that most low molecular weight metabolites can be quantified in this way, provided that their concentration *in vivo* is sufficiently high and that interference due to closely related compounds interacting with the enzyme used in the assay can be avoided.

Several scenarios can be devised for utilization of this capability in the military medical services. For example, sensors for ethanol or for various specific drug metabolites could be employed in drug screening and/or drug abuse programs. Monitoring of atropine concentrations in blood would be of aid in controlling the course of recovery of personnel from exposure to CBW agents. Similar monitoring systems could be employed in the use of other pharmacological treatments for traumatic injuries (i.e., burns, gunshot wounds). Other applications of clinical analyses using enzyme electrodes developed during our contacts with Naval personnel will be discussed in section 2.3.

Therapeutic treatments using biocatalytic methods have also been studied. Several investigators have constructed prototype artificial organs using microencapsulated or fixed immobilized enzymes (e.g. Chang, 1966, 1977). The text edited by Chang (1977) describes a wide range of possible therapeutic treatments. Among them are: a) use of L-asparaginase to attack asparagine dependent tumors; b) replacement of enzymes in genetic enzyme deficiencies; c) artificial kidney and liver for detoxification of body fluids; d) use of immobilized enzymes in a bio-fuel cell to power a cardiac pacemaker; e) use of enzymes in active transport of O_2 and CO_2 for an artificial lung; f) immobilization of antibodies for adsorption of pathogens. The detoxification and/or immunoadsorption concepts may find particular application to military medical requirements in CBW scenarios.

Other potential military medical applications of immobilized biocatalysts fall into a miscellaneous category. Stone et al (1980) have developed a strain of *B. cereus* bacteria which appears to digest non-viable eschar tissue produced by burns but not living tissue. This makes the non-viable tissue easier to remove and appears to enhance the probability of successful skin grafts. Immobilization of the cells should provide an additional degree of control and simplify management of such cases. Nair et al (1974) showed that oral ingestion of a proteolytic enzyme reduced the incidence of postoperative adhesions in rats. Again, it would appear that immobilization of these enzymes would provide an added degree of control that might limit untoward side effects.

Another long-term possible application of immobilized enzymes is their use in removing antigenic substances from the erythrocyte surface to produce a "universal blood donor". The initial phases of this project, identification and isolation of the required enzymes, are already being funded by ONR (Contract #N-00014-76-C-0269). It seems clear that, based on cost and scale factors, immobilization of the isolated enzymes would be required to make such a scheme practical. ONR has also supported a study (DDC AD D 006929) developing a dextranase active against the polysaccharides in dental plaque. Immobilization of this enzyme in a dentifrice could be prophylactic against dental caries.

2.2.4 Miscellaneous Applications

Other concepts for the use of immobilized biocatalysts in military situations cannot easily be characterized into one of the above categories. Kuan et al (134) have developed a system of immobilized enzymes wherein modified immobilized α -chymotrypsin (3.4.21.1) can be activated by light. The active enzyme then activates a second enzyme, pre-tyrosinase, which catalyzes the conversion of dopamine to the dye melanin. Overall, the system thus produces dye in response to light and therefore is an enzymic photographic process. Since it functions without silver, it may have long-term potential as a method of conserving the scarce metal.

A second interesting concept is the use of bacteria in insect control. Singer (1980) has found that strains of *Bacillus sphaericus* are toxic to several species of mosquito larva. These bacilli have long-term activity. Immobilization of the microorganisms would provide a further degree of control over this process.

2.3 Visits to Naval Facilities for Suggestions on Potential Applications of Enzyme Technology

In addition to discussions with Office of Naval Research personnel on possible naval applications of enzymes, visits were made to a number of Naval facilities. These laboratories were (1) Naval Biosciences Laboratory (NBL), Oakland, Ca., (2) Civil Engineering Laboratory (CEL), Port Hueneme, Ca., (3) David Taylor Naval Ship R&D Center (DTNSRDC) Annapolis, Md., (4) Ocean Systems Command, Ocean Sciences Division Laboratory (OSDL), Pt. Loma, Ca., (5) Naval Medical R&D Center (NMRDC), Annapolis, Md.. Personnel at each of these facilities made useful "sounding boards" regarding the potential of various concepts we presented. In addition these same people came up with a number of potentially useful applications based upon their own fields of interest.

Discussions were held with the following personnel at NBL during 2 visits to Oakland; Dr. N. Paoni and Lt. R. Arroz. Potential applications and or ideas for the use of immobilized enzymes which were brought forth by these scientists are listed below.

1. A sulphuric acid (SO_4^{-2}) sensor for air pollution monitoring.
2. Immobilization of enzymes on magnetic particle substrate for use in reactors (for ease of later separation).
3. Provide better sewage sludge aeration when using rotating disc sewage treatment devices e.g., in CHT tanks.
4. Immobilize A-zyme and B-zyme for use in the universal blood donor program.

Personnel contacted at CEL were Mr. C. Imel, Dr. D.G. Chan and Ms. S. Landon-Arnold. A wide variety of biological subjects were discussed, principally centered on pollution control systems. Three potential areas were suggested for applications to their needs. These were (1) a sensor for rapid detection of biological oxygen demand (BOD) of waste waters, (2) a technique for biodegradation of activated carbon to remove biological "crud" and (3) an indicator or indicators to show if enzymes are denatured.

A number of visits were made to DTNSRDC to discuss shipboard applications of enzymes. Personnel contacted were Dr. S. Finger and Mr. W. van Hees. Potential applications of immobilized enzymes for shipboard use centered on environmental problems, (e.g. submarine atmosphere control and monitoring and waste treatment and monitoring of effectiveness of same), chemical and biological warfare problems (e.g. agent sensors and detoxifications of exposed shipboard areas).

At the Ocean Science Division laboratory, discussions were held with Dr. S. Yamanota, Mr. M. Salazar and Mr. S. Steiner. At the time of our visit, their research efforts were concentrated on developing improved techniques for evaluating marine pollution using bioindicators such as mussels. One avenue of evaluating pollution-induced stress in such bioindicators that they were considering was to evaluate certain enzyme levels. They also suggested that a family of so called "forensic" enzyme electrodes might be developed for the detection of drugs in urine. Another probably useful suggestion was the development of an enzyme electrode for detection of CBW agents in body fluids.

The last facility visited was the Naval Medical R&D Center. Personnel interviewed were Cdr. J.F. Bates and Capt. S. Joseph. Application areas suggested were (a) disinfection of operating room and laboratory hood air, (b) removal of HLA antigens from bone marrow cells, (c) a tissue radiation damage sensor and (d) detection of medically important micro-organisms.

Overall, the personnel contacted at the various facilities reacted favorably to the concept of developing a program for utilizing immobilized enzymes for naval applications. It was the consensus that enzyme based sensors were the most likely entry point for this technology, followed by applications where controlled environment (temperature, pH, salinity) could be maintained such as medical applications. Also, it was the overall consensus that the maintenance and updating of an immobilized enzyme data base was highly desirable.

2.4 Data Base Computerization

Application of immobilized enzyme technology depends in part on the accumulation and organization of appropriate data to prevent unwarranted duplication of previous research. This can be avoided by a thorough review of such data. Journal articles, together with abstracts, reports, supplements, symposia, proceedings and books, hold a vast amount of information pertaining to the subject of immobilized enzymes that is difficult to rapidly survey. References on the topic can be most efficiently searched if brought together in a comprehensive collection and cataloged in a way which offers users multiple entry points into the classification scheme. Manual maintenance of such a system does not permit fast, reliable retrieval of the large quantity of references currently available. Further, it becomes increasingly cumbersome as the size of the collection expands. This difficulty can be overcome by the use of a computer that is designed for data string manipulation and is capable of rapidly searching through a mass of data.

Computerized access to a stored immobilized enzyme data base will facilitate the rapid selection of references on, for instance, a specific enzyme reaction or by a particular author. Abstracts accompanying these references would further enhance its value as a research tool for the Navy. Additions, deletions, and corrections can be entered via a CRT terminal. Periodic and regular printouts of the entire listing would be possible. In addition to more frequent update supplements, indexes to the references can readily be generated by a computer. Having such a system available would ensure efficient utilization of research and development funds and contribute to the coordination and planning of future enzyme research.

Data base user requirements must be spelled out more explicitly before all possible ways of meeting them can be put forward. However, some computer solutions appear to be more plausible than others. Plausible approaches include: (1) make use of a large commercial or military computer system already available for other similar purposes; (2) utilize the BioResearch mini-computer system; or (3) purchase a small mini-computer to be dedicated to the single purpose of computerizing the enzyme data base. These will be discussed below in more detail. Formalization of the user access requirements, types of data which the system would hold, and the depth of detail required for each item, should point to the most cost effective and useful system.

Each of these three computerization avenues was investigated for feasibility. We first looked into whether systems already in existence could meet or be adapted to meet our needs. For example, Lockheed Missiles and Space Company has developed the DIALOG Private File Service which offers sophisticated computer technology and services. Costs are based on the size of the

file, growth rate in terms of frequency and volume of update, and the amount of file use. There are set rates for service particulars, such as initial file loading, updates, storage, access charges, offline prints, telecommunications, file reloading and sort capabilities. Charges vary between records supplied in the Intermediate File Format (a standard format of Lockheed) and those that are not, between difference types of contracts, and between types of telecommunication options. Lockheed information systems does not maintain a data base design and production service, but will refer the user to an appropriate organization for aid in data base building. Storage would be on a high speed disc and searched through either an IBM 3032 or 3033 computer. Over 100 Lockheed system public files can be searched to provide a private data base with supplemental information.

Another available computer system which could be used efficiently is the PDP-11/60 owned by BioResearch, Inc. and operated by our Electronics Division in Ithaca, New York. An advantage of this system over the large, commercial systems such as Lockheed's, would be the vastly improved interaction between technical personnel doing the scientific literature searching and computer personnel doing the programming and data retrieval. This advantage would result in lower costs and greater convenience. Using our in-house PDP 11/60 would enable BioResearch scientific personnel to efficiently utilize the resources and capabilities of computer personnel and equipment at minimal cost.

Notable hardware features of the BioResearch PDP-11/60 are its full 248K memory and the following additional pieces of equipment:

- 3 - DEC VT 55 terminals
- 1 - DEC Rk01 dual floppy disc drive
(250K per disc)
- 1 - Pertec tape drive (DEC compatible,
1600/800 bits per inch)
- 1 - Versatec 1200A printer/plotter
(prints 1,000 lines per minute)
- 2 - RK07 Rigid discs @ 28M Bits/disk

The PDP-11/60 uses the RSX-11M multi-user operating system currently supporting the following languages:
Fortran IV Plus, Cobol, Basic and MACRO 11 Assembler

Costs for setting up an immobilized enzyme data base on the PDP-11/60 would involve the same items as in the Lockheed system. Major items would be the following:

- 1 - PDP-11/60 computer connect time
- 2 - telephone costs with a WATS line
- 3 - cost of custom or available packaged program
- 4 - cost of setting up program
- 5 - CRT terminal and modem installed in Farmingdale

Costs for the above items using the BioResearch computer will be less than if the data base was stored on another organization's computer, using their personnel.

The last alternative for computerization lies in acquiring a small computer to be devoted exclusively to the storage and manipulation of the immobilized enzyme data base. Mini-computer systems of this sort start at approximately \$25,000. An additional \$5,000 can be planned upon for programming services and an undetermined amount for key-in of the data. Generally, the memory would be half that of the PDP-11/60 and the hardware less powerful. Software ranges from floppy disc to high speed random access disc.

Deciding among these three alternatives is dependent upon further information on the internal structure and size of the data base records. Cost and convenience will be factors to weigh, as well as special features which might be available with each system.

There would be outstanding benefits both in time of efficiency and speed resulting from the computerization of the immobilized enzyme data. Timely printouts and updates would provide the most current and relevant of reference information on which to base research into Naval chemical problems.

3.0 Recommendations

Based upon the initial survey of the literature and upon our contacts with Naval personnel, several recommendations can be made as to directions in which Naval research on immobilized biocatalysts should be encouraged. First of all, it is clear that we have only begun to compile available information on biotechnology in this field. The insights gained into military applications of this technology through this initial research alone clearly indicate the advisability of maintaining and increasing this data base, and of computerizing it if possible. This would permit wide distribution of the data base and would encourage increased contact among various military facilities engaged in research and development activities bearing on this field.

General recommendations can also be made as to fields of research and development on immobilized biocatalysts in which relatively near-term benefits to the military mission(s) can be obtained. Currently, the most immediate area for development appears to be the sensing and/or removal of toxic materials, either pollutants or CBW agents. Naval laboratory personnel appeared to be more interested, at present, in the application of immobilized biocatalysts to medical and pollution-control problems rather than to CBW applications, perhaps because of the difficulty in producing a "fail-safe" biosystem, as required for CBW applications. Medical applications and pollution-control systems might therefore provide an avenue for gaining acceptance of biocatalysts as a modality for attacking Naval problems.

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GLOSSARY OF ABBREVIATIONS

AE-, DEAE - cellulose	Aminoethyl, diethylaminoethyl cellulose
AMP, ADP, ATP	Adenosine mono-, di-, or tri-phosphate
Arg	Arginine
ATCC	American Type Culture Collection
BOD	Biological oxygen demand
CNBr	Cyanogen bromide
CFSTR	Constant flow, stirred tank reactor
CySH	Cyste
DMA	Dimethyl adipimidate
FAD, FMN	Flavin adenine dinucleotide, mononucleotide
Glut.	Glutaraldehyde
Ileu	isoleucine
K_m , (K_m) app	Michaelis-Menten binding constant (apparent)
Leu	Leucine
Lys	Lysine
Met	Methionine
M-M	Michaelis-Menten
NAD/NAD ⁺ /NADH (NADP)	Nicotinamide adenine dinucleotide (oxidized/reduced form), or its phosphorylated forms
Phe	Phenylalanine
pI	Isoelectric point
Q	Flow rate
Trp	Tryptophan
U, IU	International Units of enzyme activity (unit depends on enzyme)
V _{max}	Michaelis-Menten reaction velocity constant

Appendix - Data Base Compilation

The data base given here is organized by enzyme number, as specified by the IUB Commission on Enzymes. Table 4 contains a list of immobilized enzymes encountered in abstracted references to date. As discussed earlier, our classification system also includes other categorizations of enzymes which may be of eventual use. However, it was not considered useful to include these classifications in the compilation presented herein.

TABLE 4

Immobilized Enzymes Encountered in Abstracted References

1.1.1.1	Alcohol dehydrogenase
1.1.1.27	Lactate dehydrogenase
1.1.1.37	Malate dehydrogenase
1.1.1.49	Glucose-6-phosphate dehydrogenase
1.1.3.4	Glucose oxidase
1.1.3.13	Alcohol oxidase
1.2.1.2	Formate dehydrogenase
1.2.1.3	Acetaldehyde dehydrogenase
1.4.1.1	Alanine dehydrogenase
1.4.1.2	Glutamate dehydrogenase
1.4.3.2	L-Amino acid oxidase
1.6.4.3	Diaphorase
1.7.3.3	Uricase
1.10.3.2	Phenolase
1.11.1.6	Catalase
1.11.1.7	Lactoperoxidase
2.7.1.1	Hexokinase
2.7.1.11	Phosphofructokinase
2.7.1.40	Pyruvate kinase
2.7.2.1	Acetate kinase
2.7.2.2	Carbamyl phosphokinase
2.7.3.2	Creatine kinase
2.7.4.3	Adenylate kinase
3.1.1.8	Cholinesterase
3.1.3.1	Alkaline phosphatase

TABLE 4 Con't.

3.1.26.1-5	Ribonucleases
3.2.1.1	L-Amylase
3.2.1.2	β -Amylase
3.2.1.3	Glucoamylase
3.2.1.17	Lysozyme
3.2.1.22	α -Galactosidase
3.2.1.23	β -Galactosidase
3.2.1.26	Invertase
3.4.21.1	α -Chymotrypsin
3.4.21.4	Trypsin
3.4.22.2	Papain
3.4.23.1	Pepsin
3.5.1.1	L-Asparaginase
3.5.1.5	Urease
3.5.1.11	Penicillin acylase
3.5.1.14	Aminoacylase
3.5.2.6	Penicillinase
(3.12.1.1)	Parathion hydrolase
4.1.2.11	D-Hydroxynitrile-lyase
4.1.2.13	Aldolase
4.1.99.1	Tryptophanase
4.1.99.2	β -Tyrosinase
4.2.1.2	Fumarase
4.3.1.1	Aspartase
5.3.1.5	Glucose isomerase

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
1.1.1.1	Alcohol Dehydrogenase (alcohol: NAD ⁺ oxidoreductase)	Alcohol + NAD ⁺ = Aldehyde or Ketone + NADH Cofactor: NAD	Immobilized with glut. on AE-cellulose Crosslinked with glut., NAD, and albumin	To provide source of NADH for a second enzyme reaction To show that cofactor can be immobilized in active state	Immobilized enzyme placed in packed bed. Feed solution 1.0 mM NAD, 0.2M ethanol, 0.1 M glycine. Continuous production of NADH adequate for continuous analysis in second reactor was achieved. Cost saving results because cost of purchased NADH is twice the cost of NAD and is the greatest fraction of total operating cost. Fairly simple method of generating NADH in situations where recycle of cofactor is unnecessary. NAD regenerated using O ₂ and phenazine methosulfate as electron carrier High NAD concentration leads to immobilized NAD within active site and deactivation. Optimum conc. NAD ~ 10 ⁻³ M. Membranes were used on O ₂ electrodes and showed NAD was functional. No data on membrane stability.	1
			Adsorption and crosslinking with cyanuric acid on Duolite A7 resin	To indicate industrial feasibility of reaction	Good stability over 20 hrs. at pH9, 25°C. NAD added at 2% in feed stream-hot industrially practicable due to NAD cost. Good prospect for industrial work if NAD problem is overcome.	3
			Trapped on fiber side of hollow fiber beaker (200 MW cutoff) along with acetaldehyde dehydrogenase, catalase, and diaphorase	To examine problems involved in systems requiring cofactors	Part of multienzyme system generating acetic acid from ethanol. Diaphorase used to regenerate NAD from NADH using molecular O ₂ . Catalase used to destroy any H ₂ O ₂ formed. No leakage over 10 hrs. Optimal ratio of enzyme used determined empirically. Regeneration of NAD using standard reducing agents (phenazine methosulfate, methylene blue, FMN and light) was faster than diaphorase, but requires a "tighter" cutoff on hollow fiber membrane to retain reactants - this reduces mass transport. Tight membrane needed also to retain NAD.	4

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	her Methods Results and Comments	Ref(s)
1.1.1.1	Alcohol Dehydrogenase				Cofactor requiring systems will have to be tailored to specific combination of reagents needed. In this case, perhaps a modified NAD could be used to allow a "looser" membrane. In general, semi-permeable membranes (or microcapsules) seem to allow the most flexibility if use of whole cells is ruled out.	4

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
1.1.1.27	Lactate Dehydrogenase (LDH) (L-lactate NAD ⁺ + oxido-reductase)	L-Lactate + NAD ⁺ = pyruvate + NADH Cofactor: NAD	(1) Attachment of carboxyl groups to glass modified w/γ-amino propyl groups (2) attachment of amino groups to glass modified w/succinamidopropyl groups. Covalent bonding to cellulose via chloro-triazinyl derivatives of cellulose and DEAE - cellulose Covalent coupling to porous glass, solid glass, cellulose, DEAE-cellulose, polyarylamide beads via isothiocyanate, diazotization, diimide, bromoacetyl, mercuribenzoate, and CNBr coupling agents.	To examine effects of micro environment on protein structure/function To examine immobilization method	Bound enzyme shows increased K _m for NADH, decreased for pyruvate - not diffusionally or electrically related, since + and - substitutions have same effect. Only 1 subunit of LDH was actually bound. Other units could be removed and would reattach w/good activity. Glass does not have major effects on enzyme, but secondary effects occur on kinetics, etc. Sheets stable for several months. Yield and activity not given.	7
			Covalent coupling to porous glass, solid glass, cellulose, DEAE-cellulose, polyarylamide beads via isothiocyanate, diazotization, diimide, bromoacetyl, mercuribenzoate, and CNBr coupling agents.	To examine immobilization systems.	Activity assayed by following absorbance of potassium ferricyanide as electron donor for LDH. Soluble enzyme deactivates fairly quickly @ 250. All immob. methods lost at least 79% of initial free enzyme activity. Best performances from CNBr. t _{1/2} ~ 2 weeks for remaining activity, somewhat stabilized by addition of lactate. Most efforts at immob. of LDH have stability problems perhaps because of nature of enzyme.	6

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Further Methods Results and Comments	Ref(s)
1.1.1.37	Malate Dehydrogenase	L-malate + NAD ⁺ = oxaloacetate + NADH Cofactor: NAD	Covalently coupled to dimethyl adipimidate-activated nylon tube (0.1% DMA in 30% N-ethylmorpholine in EtOH, 2 hrs., 25°C. - then enzyme in 0.1M N-ethylmorpholine buffer, pH 8.0)	To assay for glutamate - oxaloacetate transaminase (GOT) by measuring oxidation of NADH by oxaloacetate w/spectrophotometer.	NADH generated by feeding NAD and ethanol into packed column of alcohol dehydrogenase (1.1.1.1) immob. w/glut. on AE-cellulose Good calibration sensitive to 10 units enzyme/ml sample. Use of NADH generator will save money since NADH costs twice as much as NAD. Used continuously over several weeks without difficulty.	1

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
1.1.1.49	Glucose-6-Phosphate Dehydrogenase (D-glucose-6-P: NADP+1-oxidoreductase)	D-glucose-6-phosphate + NADP+ = D-glucose-6-phosphate + NADP+ Cofactor: NADP	Adsorbed together onto Sepharose beads and cross-linked w/ CNBr along with hexokinase	To compare cross-linking of 2 enzymes to one particle with separate enzymes (either soluble or on separate beads)	Linking 2 enzymes to one particle is better than separating them. 3 enzyme cascade shows this more effectively, as demonstrated by adding 8-galactosidase and using lactose as feed. Discussion of advantages and disadvantages of different arrangements for multi-enzyme systems. Local concentration gradients for 2nd stage are high, increasing rates. However, pH or other conditions may be different for each step. Coenzyme requirements may also favor multiple binding to one carrier.	11
		Covalent coupling to cellulose activated with 5-triazinyl chloride	To examine immob. method		Enzyme binds well at pH<7 not at pH>7. Binding not affected by presence or absence of substrates. Activity of bound enzyme (after washing) remained constant 3 days at 25°C, or several weeks at 20°C. pH optimum at 8.5 for both bound and free enzyme. K_m values increased for both NADP and glucose-6-phosphate by about 3-5 times for bound enzyme	8
		Physical adsorption on collodion membrane	To examine immob. method		Assay performed by following rate of NADPH production with spectrophotometer. Under all conditions, most enzyme activity disappeared from solution over about 4 hours. Adsorption rate increased with temperature. However, activity did not increase with adsorption uniformly. Earliest adsorbed enzyme was more active. Adsorbed enzyme was less temperature stable than free enzyme. Desorption is increased by increasing NADP concentration in solution.	9
		Covalent coupling to alkylated, amino-substituted nylon tube using glutaraldehyde or bisimide	To examine immob. method and its usefulness in automated analyses		All enzyme was immob. but maximum activity retained was 3.6%. Temperature stability higher than free enzyme. Very low activity retained. Enzyme is relatively fragile.	10

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Carrier Methods Results and Comments	Ref(s)
1.1.1.3.4	Glucose Oxidase (notatin, glucose, β -D-glucose oxyhydrase) Cofactor: oxygen 1-oxido reductase	β -D-glucose + O_2 = D-glucono-8-lactone + H_2O_2 Cofactor: FAD	(1) Entrapment in polyacrylamide, (2) covalent attachment to acrylamide (3) copolymerization with acrylamide by alkylation of enzyme Physical adsorption on polyethyleneimine-coated glass beads followed by cross-linking with glutaraldehyde	To compare effects of immob. methods To examine immob. method	copolymerization-activity 500U/g; Entrapment 80U/g; cross-linking 10U/g. Kinetic properties appear unchanged by copolymerization and proteins with subunits can also be handled. Copolymerized enzyme had high temperature stability Success of trapping depends on MW of enzyme - chemical attachment does not. No mention of any coenzyme (FAD) requirement or difficulty. Claims advantages for copolymerization over entrapment - no shrinkage of gel, adsorption of impurities, etc. However, immob. is more difficult. Immob. yield increased with amount of glass used, reaching maximum of 80-90%. Increased protein concentration over 1 mg/ml decreased activity. Glucose oxidase has $pI=4.2$ - binding increased with increasing pH because of electrostatic attraction between enzyme and glass. Glutaraldehyde stabilized preparation against subsequent changes in pH, ionic strength, or washing. Thermal stability increased by ~20X. Use of non-porous carrier limits amount of enzyme bound, but increases % of active enzyme. High yield obtained with high efficiency will be good for expensive enzymes. Not clear how useful this might be for "fragile" enzymes.	12
			Covalent coupling to either porous glass or silica-alumina with O, 2, 8% calcined nickel coating via isothiocyanate.	To examine immob. methods	Activity assayed via O_2 electrode. Gentle mixing to avoid glass breakage essential. Si-Al support with 0% Ni had 50% lower initial activity. This increased 75% when 2% Ni coating was used but was not further increased using 8% Ni. Freezing at -230 destroyed enzyme but storage at 5°C was possible with half-life greater than 1 month. Activity increased with decreasing glass diameter indicating diffusional limitations. Half-life somewhat longer on glass. Use of adsorption alone gives about 7% and 14% of reference activity on glass and Si-Al. IE used in reactor for 1 month (24 hrs/day) with good stability.	6

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/Co-factors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
1.1.3.4	Glucose oxidase		Solid phase enzyme immob. between polyacrylamide and polycarbonate membranes	To measure glucose levels as input to an artificial pancreatic 8-cell	Electrode senses production of H_2O_2 by enzyme. Sensitivity > 15nA/20 mg/dL glucose. 90% time response 50±3 sec. Drift ~ 2%/hr. Range: 0-600 mg/dL. Sensors can be calibrated by internal addition of glucose standards. No details of immob. methods given.	14
			Enzyme covalently bound to polyacrylamide - method not given.	To produce a glucose electrode	Enzyme layer placed over electrode polarized to +600mV to detect H_2O_2 . Reproducibility to within 2% based on either steady-state or initial rate measurements. Relation between current or rate is linear up to ~ 15mM glucose. Stability of several weeks observed.	15
			Mixed with albumin and cross-linked with glut.	To develop sensor for glucose	Cross-linked enzyme placed over polarographic O_2 electrode and secured by cloth and O-rings. Glucose detectable to 1.0 mg%. Electrode stable over 4 months. This method found more sensitive than detection of H_2O_2 production because of side reactions consuming H_2O_2 .	16
			Microencapsulation: aqueous enzyme solution emulsified in ethyl cellulose and/or polystyrene in benzene in 5-6 secs. by machine. This was poured into 2nd phase, H_2O w/emulsifier (polyvinyl alcohol or sodium lauryl sulfate and stood for 2-3 hrs. to allow benzene to evaporate.	To study immob. method	Benzene did not denature enzyme. Good emulsion obtained. Stability of 2nd emulsion most important. Capsulation yield up to 70% depending on emulsifier and composition of 2nd phase (added salts increase yield). Osmotic balance must be retained during encapsulation or pressure will break capsules, reducing yield. If osmosis is out of capsules, capsules shrink and activity is reduced. Microencapsulation is a delicate immob. process compared to several others.	18

I...B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
1.1.3.4	Glucose oxidase		(1) Casting and drying of collagen-enzyme mixture with or without cross-linking with glut. (2) electrodeposition of collagen-enzyme mixture (3) impregnation of enzyme into collagen membrane w/o cross-linking w/glut. Covalently coupled to porous glass by diazotization reaction	To examine collagen as enzyme carrier	O ₂ electrode used to measure activity pH and temp. vs activity profiles same as free enzyme. Bound enzyme is slightly more stable at high pH and temp. Apparent $K_m = 0.072 M$, about 3X that of free enzyme, indicating some steric hindrance. Fairly general and cheap immob. method.	19
				Use as glucose detection and measurement system.	Glass beads packed in miniature column operated in either endpoint or kinetic mode by measuring rate of reaction or final conc., depending on whether system is run in batch or continuous mode. Sensitive down to $\sim 10^{-5} M$. Time $\sim 30-60$ secs. for discrete samples, 20-30 secs. for continuous. Residence time in reactor $\sim 3-5$ secs. Discussion of use of coenzyme-requiring enzyme for analysis. Authors feel best method is with soluble enzyme and soluble, polymer-linked coenzyme in hollow reactor.	20
			Adsorbed onto porous TiO ₂ , Al ₂ O ₃ or combinations along with catalase (1.11.1.6)	To examine synergistic effects of simultaneous immob. of both enzymes.	Reaction rate monitored with conductivity meter - no explanation given here of how this might work. Column as prepared is flow limited up to 350 ml/hr. At lower flow, activity was increased by addition of H ₂ O ₂ to 0.0075% indicating facilitation of glucose oxidase activity. Small pore (175, 220Å) preparations unstable. Related to enzyme molecule size. Catalase appears to protect glucose oxidase from H ₂ O ₂ .	17

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
1.1.3.13	Alcohol Oxidase	$\text{RCH}_2\text{OH} + \text{O}_2 \rightarrow \text{RCHO} + \text{H}_2\text{O}_2$ Cofactor: FAD	Enzyme adsorbed onto porous surface and trapped between two semi-permeable membranes (e.g. cellulose, cellophane, dialysis membrane)	To develop an alcohol sensor	Platinum anode polarized at 0.6V used to measure H_2O_2. Tubular anode used so that increased O_2 can be provided for reaction. Polarogram has stable plateau at 0.6-0.8V. Current without alcohol $\sim 0.03 \mu\text{A}$. Current is proportional to conc. H_2O_2 up to about 500 nM H_2O_2 and increases with temperature. Gas or liquid alcohols can be used. Sensitivity decreases with increasing MW. Electrode not stable overnight. Cofactor requirements, not discussed here, and stability are problems.	127

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Cover Methods Results and Comments	Ref(s)
1.2.1.2	Formate Dehydrogenase	Formate + NAD ⁺ = CO ₂ + NADH Cofactor: NAD	NAD ⁺ covalently attached to dextran by ethylenimine/carbodiimide. Dextran-NAD ⁺ and enzymes (formate and alanine dehydrogenase recirculate in loop behind ultrafiltration membrane.	Test of use of modified coenzymes in recirculating reactor with ultrafiltration membrane	Reaction monitored w/polarimeter. Without UF membrane, most of signal is from NAD ⁺ . When UF membrane is added, no signal from NAD ⁺ in product, only slow increase from alanine - successful trapping and recycle of NAD ⁺ . Covalent bonding of NAD ⁺ is simple way to keep coenzymes around. No information on stability	22

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Results and Comments	Ref(s)
1.2.1.3	Acetaldehyde Dehydrogenase	Aldehyde + NAD ⁺ = Acid + NADH Cofactor: NAD	Trapped on fiber side of hollow fiber beaker (200 MW cutoff) along with alcohol dehydrogenase, diaphorase, and catalase	To examine problems involved in systems requiring cofactors	Part of multienzyme system generating acetic acid from ethanol. Diaphorase used to regenerate NAD from NADH using molecular O₂. Catalase used to destroy any H₂O₂ formed. No leakage over 10 hrs. Optimal ratio of enzymes used determined empirically. Regeneration of NAD using standard reducing agents (phenazine methosulfate, methylene blue, FMN + Light) was faster than diaphorase, but requires a "tighter" cutoff on hollow fiber membrane to retain reactants - this reduces mass transport. Tight membrane needed also to retain NAD. Cofactor requiring systems will have to be tailored to specific combination of reagents needed. In this case, perhaps a modified NAD could be used to allow a "looser" membrane. In general, semi-permeable membranes (or microcapsules) seem to allow the most flexibility if use of whole cells is ruled out.	4

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
1.4.1.1	Alanine Dehydrogenase	Pyruvate + NAD ⁺ covalently attached to dextran by ethylenimine/carbodiimide. → Alanine + NAD ⁺ + H ₂ O Cofactor: dehydrogenase NAD ⁺	Dextran-NAD ⁺ and enzymes (alanine and formate) recirculate in loop behind ultrafiltration membrane.	Test of use of modified coenzymes in recirculating reactor with UF membrane	Reaction monitored w/polarimeter - NAD ⁺ and alanine are active species. Without UF membrane, most of signal is from NAD ⁺ . When UF membrane is added, no signal from NAD ⁺ in product, only slow increase from alanine - successful trapping and recycle of NAD ⁺ Covalent bonding of NAD ⁺ is simple way to keep coenzymes around. No information on stability.	22

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
1.4.1.2	Glutamate Dehydrogenase	L-Glutamate + H ₂ O + NAD = 2-oxoglutarate + NH ₃ + NADH Cofactor: NAD	Crosslinked with glut. on aminoethyl cellulose	To examine enzyme kinetics in a packed-bed reactor	Activity 14% of free enzyme K_m increased by about 5x. Sigmoid kinetics observed in recycling column, with apparently (?) strange inhibitions due to product. Apparently, immob. enzyme can give strange kinetics due to diffusion limitations and catalyst inhibitions which may be different than in free enzyme system.	23

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
1.4.3.2	L-Amino Acid Oxidase	$\text{NH}_3\text{-CHR-COO}^- + \text{H}_2\text{O}_2 + \text{O}_2 \rightarrow \text{RCO-COO}^- + \text{NH}_3 + \text{H}_2\text{O}_2$	Entrapped in polyacrylamide gel on dacron or nylon net	To measure total amino acid conc.	Net placed over cation-selective electrode responsive to NH_4^+. Electrodes with higher enzyme conc. more stable. pH 8.5 optimum. Stability generally decreased with increasing amino acid conc. Lower limit measurable 10^{-4} M. No discussion on selectivity but this probably limits utility as with most cation-selective electrodes. Probably better w/O_2 electrode.	24
			Mixed with albumin and cross-linked with glut.	To develop sensor for amino acid	Cross-linked enzyme placed over polarographic O_2 electrode and secured by cloth and O-rings. Primary effectiveness on Met, Leu, Phe, CysH, Ileu, Lys, because of different reaction rates with enzyme. Detectable to as low as 1 mg%. Electrode stable over 4 months. This method appears more sensitive than the detection of H_2O_2, because side reactions use up H_2O_2. Simple, stable electrode preparation but not specific for any or all amino acid(s).	16

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
1.6.4.3	Diaphorase	NADH + lipoamide = NAD ⁺ + dihydro-lipoamide	Trapped on fiber side of hollow fiber beaker (200 MW cutoff) along with alcohol dehydrogenase, acetaldehyde, and catalase	To examine problems involved in systems requiring cofactors.	Part of multienzyme system generating acetic acid from ethanol. Diaphorase used to regenerate NAD from NADH using molecular O₂. Catalase used to destroy any H₂O₂ formed. No leakage over 10 hrs. Optimal ratio of enzymes used determined empirically. Regeneration of NAD using standard reducing agents (phenazine methosulfate, methylene blue, FMN + light) was faster than diaphorase, but requires a "tighter" cutoff on hollow fiber membrane to retain reactants - this reduces mass transport. Tight membrane needed also to retain NAD. Cofactor requiring systems will have to be tailored to specific combination of reagents needed. In this case, perhaps a modified NAD could be used to allow a "looser" membrane. In general, semi-permeable membranes (or microcapsules) seem to allow the most flexibility if use of whole cells is ruled out.	4

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Further Methods Results and Comments	Ref(s)
1.7.3.3	Uricase (urate oxidase)	urate + O ₂ → products	Electro codeposition w/collagen in rectangular cell with anodes at sides, central cathode. I ~ 4mA/cm ² , 5°C, 2 min pH 3.8-4.5 or 10.4 Collagen ~ 0.45% by weight	To examine immob. system.	Clamped over O ₂ electrode to measure uric acid conc. Collagen deposits on cathode pH 2.5-5.3, anode 9-12. Activity retained ~ 43%. Can clamp membrane over O ₂ electrode as uric acid sensor with good linearity over 0-1.0uM. Before deposition, salts must be washed out of enzyme solution to get good membrane.	25

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
1.10.3.2	Phenolase (Laccase) Benzenediol: Oxygen oxidoreductase	4 Benzene- diol + O ₂ = 4 Benzenediol + 2H ₂ O Cu	Entrapment of enzyme solution into emulsion microspheres formed by shear (conditions not given) forming water in oil in water emulsion. Oil phase is 2% Span 80, 3% ENJ-B029, 95% S100N; a detergent, amine and hydrocarbon, respectively.	To study the properties of liquid membrane emulsions	Phenol is removed from surrounding. Other substrates which could not penetrate membrane were not reacted. Very little enzyme leach. Initial study of liquid membranes as method for enzyme immob.	26
		Adsorption onto carbon-black electrodes	To examine use of adsorbed enzyme to accelerate electrochemical reactions	Laccase accelerates the O ₂ reduction (higher V at constant current). This effect can be eliminated by various enzyme inhibitors. Apparently the enzyme serves as an electron transfer agent making reaction almost ideal. Electrode was stable for up to 50 hrs. Direct electron transfer via enzyme demonstrated. In biological conditions this may be useful to cut energy demand of systems with high overvoltages.		27

I.C.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
1.11.1.6	Catalase	$H_2O_2 + H_2O \rightarrow O_2 + 2H_2O$	(1) Dispersion and complexation in collagen membrane and cross-linking of collagen with glut.; (2) Physical impregnation of enzyme into swelled membrane; (3) electrocodeposition of collagen and enzyme Physical adsorption onto oil microdroplets	To show various possibilities available w/collagen, a cheap membrane material To examine activity of enzyme	Method (1) needs pH 2-4.5 or 8.5-12 for 15-20 min. can be bad for some enzymes. Then go to (2) or (3) Collagen can be used for all enzymes - not necessarily better than artificial substances. No discussion of activity and/or stability. Enzyme appears to be partially denatured by changes in secondary and tertiary structure. If denaturation is incomplete, desorption reactivates enzyme. Increased adsorption strength correlates with decreasing activity. Indicates problems with using adsorption as an immobilization method.	33
			Adsorbed onto porous TiO_2 , Al_2O_3 or combinations along with glucose oxidase	To examine synergistic effects of simultaneous immobilization of both enzymes.	Reaction rate monitored with conductivity meter - no explanation given here of how this might work. Column as prepared is flow limited up to 350 ml/hr. At lower flow activity was increased by addition of H_2O_2 to 0.0075% indicating facilitation of glucose oxidase activity. Small pore (175,220Å) preps. unstable. Related to enzyme molecular size. Catalase appears to protect glucose oxidase from H_2O_2 , thus resulting in increased conversion of glucose. Indicates possibilities of multiple enzyme systems to protect enzymes from product inhibition, etc.	17

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
1.11.1.6	Catalase		Variety of techniques: entrapment, absorption on DEAE - cellulose or cheesecloth followed by cross link with glut., crosslinking of crystalline enzyme with glut. Microencapsulation in buffered hemoglobin solution into collodion microcapsules	To examine use of enzyme to remove H_2O_2 used in milk treatment To study use of microencapsulated enzymes for treatment of enzyme deficiency.	Enzyme loaded into packed-bed reactor. Enzyme stable. Best initial activity from DEAE - cellulose and cross-linked crystals, however activity decreased rapidly after 40 min. Possibly due to high conc. of H_2O_2. May be overcome by different immob. techniques or different reactor. Acatalasemic mice used as in vivo assay. Mice immunized by injections of beef catalase died on injection of large amount of free catalase, but not from equivalent amount in microcapsules. Capsulated enzyme has $t_{1/2}$ of 4.4 days in vivo; free enzyme, 2.0 days. Encapsulated enzyme was more effective in removal of perborate from blood of immunized mice. K_m is about equal for both forms of enzyme, but V_{max} is 5 times larger for free enzyme, due to diffusional limitations. Indicates problems to be faced in enzyme therapy and solutions available by immob.	29 30
			Electrocodeposition with collagen in rectangular cell with anodes at sides, cathode in center. $I \sim 4mA/cm^2$, $50^\circ C$; 2 min. pH 3.8-4.5 or 10.4. Collagen $\sim 0.45\%$.	To examine immob. method	Collagen deposits on cathode @ pH 2.5-5.3, anode 9-12. Activity of enzyme $\sim 95\%$. Can be clamped over O_2 electrode as H_2O_2 sensor with good linearity but high zero current. Conc. of H_2O_2 $> 1.5mM$ causes non-linearities. Salts in enzyme solution must be dialyzed out first to retain activity. Simple, apparently general immob. method. Current, pH allow control of immob. process.	25

I.C.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Resu..s and Comments	Ref(s)
1.11.1.6	Catalase		Codeposition with collagen into membrane (followed by cross-linking w/glut.).	To examine reaction kinetics in immob. enzyme system.	Enzyme rolled into spiral membrane form, with H ₂ O ₂ assayed iodometrically. Cross linking w/glut. reduces activity from 26.3% of free enzyme level to 3.5% - not good compared to gain in stability produced by cross-linking. Uncross-linked membranes stable 5 months at 40C. Inactivation of enzyme was faster at higher conc. of H ₂ O ₂ . Reactivation was produced by incubation with phosphate buffer, and increased with incubation time. Inactivation tends to make use of immob. catalase less feasible, although regeneration shown is hopeful.	32
			Microencapsulation in collodion with or without inclusion of hemoglobin and/or cross-linking with glut.	To examine enzyme properties under different immob. conditions.	Activity measured by perborate. Stability increased by both addition of hemoglobin and by cross-linking. However, cross-linking decreases activity. "Inert" protein seems to stabilize microcapsule preparations. This may be generally helpful.	34
			Covalent coupling via isothiocyanate to glass, alumina, or silica-alumina coated w/nickel oxide.	To examine immob. method	Assay by O ₂ electrode beginning w/H ₂ O ₂ in deoxygenated solution. Activity decreased w/ increasing particle size. Increased nickel coating increased binding. Composition of support (% Si, Al) affected stability noticeably only in the smaller particles (below 250μ). In large particles, diffusional restrictions within particles due to pore size predominate. In smaller particles, specific effects due to support composition are important.	31

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
1.11.1.6	Catalase		(1) Casting and drying of collagen enzyme mixture with or without cross-linking w/glut. (2) electrodeposition of collagen/enzyme mixture (3) impregnation of enzyme into collagen membrane, with or without cross-linking w/glut.	To examine collagen as enzyme carrier	Activity measured by iodometric titration. About 80% activity lost by washing membrane made by method (3) with swelling of membrane by solvent before adsorption. All activity lost without swelling. No information on other immob. methods. Casting of mixture seems to be easiest way to make membrane. Fibers with immob. enzymes can also be produced this way.	19

I.C.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
1.11.1.7	Lactoperoxidase	Donor +H ₂ O ₂ + oxidized donor +2H ₂ O	Covalently bonded to Sepharose - 4B activated w/ CNBr.	To examine the effect of a peroxidase column as a bactericide combination w/H ₂ O ₂ and iodide	Immob. enzyme incubated w/organisms in tubes at 25°C for 30 min. Up to 85% of E. coli and S. aureus killed using lactoperoxidase. Other peroxidases were less effective. Relatively high conc. of enzyme required. Economics of process are suspect due to enzyme costs.	35
		"	Covalent coupling to poly(4-methacryloxybenzoic acid) via N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline	To examine immob. method	Good immob. No properties given.	126
		"	Immob. on CNBr - activated sepharose	To examine use of immob. enzymes in milk treatment	Placed immob. enzyme in treatment tubes w/H ₂ O ₂ , KI and either E. coli or S. aureus for 30 min. then plated and counted live organisms. 85% kill of S. aureus and E. coli obtained using 0.026 units of enzyme. Higher activity than other peroxidases tried even though a lower amount of enzyme was used.	29
	Horse-radish Peroxidase		Immob. on CNBr-activated sepharose	To examine use of immob. enzymes in milk treatment	Placed immob. enzyme in treatment tube with H ₂ O ₂ , KI, and either E. coli or S. aureus for 30 min., then plated and counted live organisms. 40% kill of S. aureus, 5% of E. coli - apparently not a good bactericidal enzyme.	29

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
2.7.1.1	Hexokinase	Glucose + ATP = Glucose-6-phosphate + ADP Cofactor: ATP	Covalent coupling to alkylated amino-substituted nylon tube using glut. or bisimide	To examine immob. method and its usefulness in automated analysis	All enzyme applied to tube was immob. but only 4% remained active. In these experiments activity decreased with increasing amount of enzyme immob. Temperature stability higher for fixed than for free enzyme. Enzyme molecule is fragile - low activity retained.	10
			Covalently bonded to polyethylenimine treated silica which is bonded to aminoethyl cellulose	To examine properties of immob. enzyme	Temperature and pH vs. activity profile similar for fixed and soluble enzymes. K_m values are fairly close to that for free enzyme in batch reaction. In continuous system, K_m of bound enzyme is about 10 times that of free enzyme (due to diffusional resistance, etc.). Immob. enzyme has better stability than free enzyme - 50% activity retained vs. 10% after 2 weeks at 40C.	36

I.C.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
2.7.1.11	Phosphofructokinase	ATP + D-fructose-6-phosphate = ADP + D-fructose-1,6-biphosphate Cofactor; ATP	Adsorbed on Sepharose and cross-linked w/CNBr	To examine allosteric effects on activity of immob. enzyme.	At pH 6.9, immob. enzyme had maximum activity for ATP conc. between 0.12mM and 0.40mM. Maximum activity for free enzyme was at 0.03mM. Immob. enzyme was more stable. Bound enzyme also does not show allosteric effects of other compounds known to have such effects on free enzyme. This enzyme is known to have several allosteric sites. Immob. apparently inactivates the allosteric effects.	37

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
2.7.1.40	Pyruvate Kinase	ATP + pyruvate = ADP + phosphoenolpyruvate Cofactor: ATP	Covalent bonding to cellulose via chloro-triazinyl derivatives of cellulose and DEAE-cellulose	To examine immob. method	Sheets stable for 1-2 months. Yield and activity not given.	5

I.L. J. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
2.7.2.1	Acetate Kinase	Acetyl phosphate + ADP = Acetate + ATP Cofactor: ATP	Covalently coupled to CNBr-activated Sepharose	To produce ATP on a pilot-plant scale for use in a pilot process enzymatically synthesizing the antibiotic Gramicidin S.	Enzyme operates along with adenylate kinase (2.7.4.3) which produces ADP from AMP and ATP. Net reaction is production of acetate and ATP from AMP and acetyl phosphate. Scheme is thought to have good general applicability for ATP synthesis. Acetyl phosphate can be made fairly cheaply from ketene and phosphoric acid. Either ADP or AMP can be used as feed. Some activity is retained up to 6 weeks - major problem is oxidation of SH groups. Overall thermodynamics of reaction is favorable. Small column (Q=3 ml/min) generator $\sim 1/8$ ATP/hr., about 6-9 times as much ATP as in feed. This seems like a fairly good system for making ATP, since acetyl phosphate can be made inorganically and ADP (or AMP) can be recycled.	38

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
2.7.2.2	Carbamyl Phosphokinase	$\text{NH}_2\text{COHPO}_4^- + \text{ADP} + \text{NH}_2\text{COO}^-$	Alkylamine glass derivatized with glut. followed by addition of enzyme	To study use of this enzyme for continuous ATP regeneration	Carbamyl phosphate was either added directly or formed in solution from cyanate and dihydrogen phosphate. About 26% of enzyme activity was retained after immob. 16% of initial activity lost over 14 days @ 40°C. Second fairly good looking ATP production reaction. Carbamate product can be recycled via NH_3, CO_2.	39

I...B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Author Methods Results and Comments	Ref(s)
2.7.3.2	Creatine Kinase	ATP + crea- tine + ADP + phospho- creatine ATP	Covalent bonding to cellulose via chloro-triazinyl derivatives of cellulose and DEAE-cellulose	To examine immob. method	Sheets stable several months. Yield, activity not given.	5

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
2.7.4.3	Adenylate Kinase	AMP + ATP = 2ADP	Covalently coupled to CNBr-activated Sepharose	To produce ATP on a pilot-plant scale for use in a process enzymatically synthesizing the antibiotic Gramicidin S.	Enzyme is used along with acetate kinase (2.7.2.1) which transfers phosphate from acetyl phosphate to ADP. Net reaction is production of acetate and ATP from AMP and acetyl phosphate. Scheme is thought to be generally applicable to ATP synthesis. Acetyl phosphate can be made fairly cheaply from ketene and phosphoric acid. Either ADP or AMP can be used as feed. Some activity retained up to 6 weeks - major problem is hydrolysis of -SH group. Overall thermodynamics of reaction are favorable. Small column (0-3 ml/min) generates ~1 g ATP/hr, about 6-9 times as much as ATP as required in feed. Should be a fairly cheap system of making ATP, since acetyl phosphate is made inorganically and ADP or AMP can be recycled.	38

I. B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
3.1.1.8	Choline- terase	acetylcholine choline + acetyl acid Cofactor: None	Entrapped in starch gel on ure- thane foam	To detect choline- sterase inhibitors in watersamples	Enzyme is exposed to inhibitor and then to substrate. Inhibition causes change in level of hydrolysis products which is monitored polarographically. This report describes conversion of original fixed, automatic model to a completely portable, semi-manual AC/DC operable device. Field testing of device indicated fairly good resistivity (<1 ppm) maintained to a variety of organophosphates. (1) Air monitor - air + acetyl thiocholine liquid pumped through gel. Thiocholine normally hydrolyzed then reduced at const. voltage. If inhibition occurs, less thiocholine available, voltage increases + alarm. (2) Water - alternate cycles of water for testing and acetylthiocholine used since otherwise substrate conc. is too low. (3) Field kit - add acetylcholine and indicator to enzyme paper after exposure - look for color. Can detect down to 0.1 ppm of some organophosphate insecticides. Later papers by same authors available.	133
			(1) Entrapped in starch gel on poly- urethane foam (2) covalently bound to ion ex- change paper w/cross linking agents	To detect presum- ably toxic inhibi- tors of choline- terases	(1) Air monitor - air + acetyl thiocholine liquid pumped through gel. Thiocholine normally hydrolyzed then reduced at const. voltage. If inhibition occurs, less thiocholine available, voltage increases + alarm. (2) Water - alternate cycles of water for testing and acetylthiocholine used since otherwise substrate conc. is too low. (3) Field kit - add acetylcholine and indicator to enzyme paper after exposure - look for color. Can detect down to 0.1 ppm of some organophosphate insecticides. Later papers by same authors available.	40
			Enzyme is absorbed into an aluminum hydroxide gel via coprecipitation. Gel is resuspended in starch and ap- plied to urethane foam	To develop a de- tector of choline- sterase inhibitors.	System uses polarographic detection of hydrolysis product to detect change in enzyme activity due to inhibition. Activity of coprecipitated enzyme more stable than that made by starch entrapment alone. Also pads made in this way are more resistant to washout of enzyme. Activity of enzyme is fairly uniform from pad-to-pad, so detector sensitivity will remain constant.	131
			Impregnated or discs - method not given	To differentiate between reversible and irreversible cholinesterase inhibitors	Detection to proceed by a) exposing enzyme to inhibitor, producing color change; b) exposing inhibited enzyme to reactivator. Reactivations of up to 70% have been achieved. However, attempts to develop a more stable chromogenic substrate were not yet successful. Quarterly progress report - development continuing.	132

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
3.1.3.1	Alkaline phosphatase	R-phosphate R + phosphate R is a monoester	Coupled to substituted silanized porous glass. (790 Å ± 50 Å pores) Physical adsorption	To examine porous glass as enzyme carrier. To study enzyme kinetics	Activity assayed spectrophotometrically w/para-nitrophenyl phosphate pH optimum between 9-10, same as free. $K_m \sim 6.1 \times 10^{-4} M$ (free enzyme value not given) Early paper on porous glass immobilization. Enzyme assayed spectrophotometrically via p-nitrophenyl phosphate. Theory: Relations derived associating reaction rate w/surface and bulk substrate concentration and apparent K_m for reactions w/product inhibition. Expt: Adsorbed enzyme less stable than free enzyme at 60° and 80° C. pH-activity profile of adsorbed enzyme indicates that local pH is lower than bulk pH. K_m values are higher than free enzyme by 25-30 times, and 10 times those calculated without including a stagnant layer. Estimated thickness of stagnant layer ~ 42-66μ. Solution is steady-state and assumes substrate on both sides of membrane and that product concentration remains zero outside - only at short times after start.	41 42

I. B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	her Methods Results and Comments	Ref(s)
3.1.26	Ribonuclease	Endonuclease removing one residue from RNA polymer	Immobilized on ceramic supports or on glass fiber filters - method not given	To examine possibility of aerosol disinfection via enzyme filters	Active enzyme filter compared to heat-denatured enzyme filter. For air residence times of about 1 sec., viral concentrations of two influenza strains and one Coxsackie virus strain were typically decreased to 1% of inlet level. Enzyme life may be up to 6 months. Potentially useful in disinfection systems.	21

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
3.1.26.2	RNAase	Endonuclease cleaving 5'-phospho-monoester specific for 0-methylated RNA "	(1) Enclosure in ultrafiltration cells; (2) Adsorption onto silica. In both cases, enzyme is reacted with dimethyladipimate to intra-link lysine groups - in (1) before, in (2) after immob.	To see whether activity + stability can be retained or improved by intra-molecular cross-linking away from active site	Activity improved by DMA to 160% of original. Stability at 65°C greatly improved (@ 48 hours still 30% original activity) Note: active site lysine residue protected by phosphate- method not given. Good trick for improving stability of enzymes: requires detailed knowledge of primary, 2nd, 3rd level structure to employ reagents effectively.	128

I...B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
3.1.26-27	RNAase	Endoribo- nuclease	Adsorption onto various resins	To compare differ- ent resins for enzyme immob.	Activity varies from 2.1-37.4 IU/ml resin. Best is DUOLITE A-7 (phenolformaldehyde). Characteristics thought good for industrial reaction.	3

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
3.2.1.1	L-Amylase	Hydrolysis of 1,4-residues of glycossides	Covalent coupling to poly (4-methacryloyloxybenzoic acid) via N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline	To examine immob. method	Good immob. No properties given.	35
		"	Electrocodeposition w/collagen. Collagen in rectangular cell w/2 anode @ sides and central cathode. DC operation.	To examine immob. enzyme method and possible applications.	Salts in solution prevent membrane formation, so enzyme solutions must be dialyzed vs distilled H ₂ O. Current= 2-4 mA/cm ² for 2 min. Electrolyte cooled w/ice. Collagen membranes form for 2.5< pH<5.3 and 9< pH<12 (pzc ~7-8). For pH>7, membrane does not stick to electrode (anode). Enzyme migrates ~ same as collagen. Act ~ 55%. Fairly complex method.	25
			Adsorption of pure and acylated enzyme onto millipore filters	to examine behavior of acylated enzyme in comparison w/free and immob. enzyme	K _m not affected by acylation. V _m decreases w/acyl chain length and amount of acyl. Heavily acylated groups form suspensions. pH opt ↑ with chain length and amount acyl. T stability increased by acylation. Acylated enzymes more easily immob. on filter. Heavily acylated enzyme bonds hydrophobically. Activity ↓ 40-50% on immob. Packed in column with starch feed- acylated enzyme more stable. Not a good method of immob. Also not clear that the derivatization used gives complex typical of immob. enzyme.	44

I...B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
3.2.1.1			(1) Adsorbed to Duolite + cross-linked w/glut. (2) cross-linked w/glut, to etched (HCl) nylon tubing (3) cross-linked w/glut. to acrylamide treated cotton. (4) Bound to porous glass (arylamminated) with or without glut. (5) Bound to silanated NiO.	To treat starchy waste waters discharged from paper mills	(1) No activity lost over 10 days. Stored for 2 mos. @ 50°, then restarted w/no difficulty. Some plugging solved by washing. (2) 10 days ok. Stored at room temperature 3 mos. Restart ↓ 25% but stable. (3) 80% loss in activity after 400 hrs. (4) Ok over 94 hrs. in CFSTR. In column, activity ↓ 50% over 10 days. Above on test starches. Nylon and Duolite resin seem to be good in test samples (readily degrade starch). One of few papers so far on waste treatment applications of enzyme immob.	45

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
3.2.1.2	β -Amylase (Diastase)	Hydrolysis of 1,4- α -D-glycosidic linkages in polysaccharides to remove maltose units from non-reducing ends of chains	Covalently bonded to oxo-agarose using cyclohexyl isocyanide	To examine immob. procedure	Activity toward starch measured. 40% of free enzyme activity @ pH 4.8. Free enzyme w/5% starch @ 60° loses activity in 25 hrs. at 60°, while immob. enzyme stays 95% active	46

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	References
3.2.1.3	Glucoamylase Amyloclucosidase	Hydrolysis of terminal 1,4 linked α -D-glucose residues successively from non-reducing ends with release of 8-D-glucose (Maltose + glucose)	Covalently coupled to silanized or arylaminated glass using diazotization or glutaraldehyde. Some glass was coated with TiO_2 , ZrO_2	To examine system for commercial application in starch $\rightarrow$ dextrose processes.	47 Compared PF, CSTR reaction systems. pH: free enzyme peaks 3.5-5.0. Azo - immob. enzyme @ 5; glut. @ 4. K_m for azo ~ free, for glut. ~ 2 times free. $t_1/2$: Coated glass longer (20-70 days) than uncoated (4-40 days). Temperature: about the same as free enzyme. Models for reactor operation using exponential decay in plug flow or CFSTR system did not fit data well.
			Entrapment in gels made by radiation polymerization. Gels made from: (1) acrylamide (2) dimethylacrylamide (3) 2-hydroxyethylacrylate (4) Na acrylate (5) N-vinylpyrrolidone (6) polyvinylalcohol	To examine immob. method.	48 Glucose measured by glucostat. At lower radiation levels (~1 mrad), gelation in acrylamide occurred w/leakage. Leakage stopped at 2mrad. Weight of gel decreased with ~40% of activity retained. In DMA, 53% activity. 2-HEA gelled at 4mrad w/leakage. 5-6 mrad. needed to prevent leakage, but low activity (18%) obtained. With (5) gelation at 1 mrad, but 2.9 needed to stop leakage - activity 45% - depends on enzyme. PVA gelled at 4mrad, w/o leaks @ 5mrad. Higher activity in PVA (49% vs 24%) w/more intense rad for shorter time. Need low enzyme conc. to prevent leakage. K_m for gel from (5) ~ 2.8 mM compared to 1.1 mM for free enzyme. Activity on dextrin decreases w/increased MW of dextrin, as expected. For MW 4000, same activity as on maltose. pH profile similar to free enzyme. Indicates limits of gel-entrapment - MW of substrate <4000 or less is needed for good activity w/this method.

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Co-factors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
3.2.1.3	Glucoamylase		Entrapped in radiation polymerized polyacrylamide Covalently coupled to silanized ceramic support w/glut. Supports were different compositions of Si, Ti, Zr, Al, Mg oxides.	To investigate immob. method Scale-up studies of system for cornstarch hydrolysis + glucose.	Shift in pH opt - 1.0 unit. Opt. T slightly higher. Relatively mild method for immob. subject to all entrapment problems. Enzyme packed in column, activity assayed w/glucostat. 50 Zr-containing supports not as good as others. $t_{1/2}$ dec. with inc. temperature. Longest $t_{1/2}$ = 113 days @ 50°C. In pilot plant, (0.98 cu. ft. reactor), 89% of enzyme actively immob. by coupling in situ (circulating enzyme and glut. through reactor). Column run at 37-39°, started up with CHCl_3 as disinfectant: Constant activity over 70 days, yielding 89-90% glucose from 30% starch solutions-pH 4.5. 1200 lbs glucose/day produced at flow rate of 1100 ml solution/min. Some contamination occurred, reversible via CHCl_3, wash of calcium, with no effect on enzyme. 10 lb/yr plant can be competitive w/current processes using 7 parallel columns with staggered changeover as activity dec. ~ 25% of enzyme applied was fixed. No fixation w/o reduction step	49
			Covalently coupled to agarose using thiol agarose to reduce S-S enzyme bridges to -SH and pyridine-disulfide agarose to react with reduced enzyme	To study immob. method		46

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
3.2.1.1.7	Lysozyme Muramidase Mucopolysaccharide hydrolase	Hydrolysis of 1,4- β linkages between N-acetylmuramic acid and 2-acetamido-2-deoxy-D-glucose residues in mucopolysaccharides or muropeptides	Cross-linking to 4-vinylbenzoic acid-styrene copolymer with N,N' -Carbonyldiimidazole in DMF solution.	To show that non-aqueous immob. can be done.	Enzyme survived well - pH optimum $\downarrow$ 0.4 units. Bound lysozyme had about 40% of native activity. Stability after 72 hours. proved that non-aqueous immobilization was possible. May have use in altering activity and stability profiles of immob. enzymes.	51
			Covalent coupling to poly (4-methacryloxybenzoic acid) via N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline	To examine immob. method	Good immob. No properties given,	126
			(1) casting and drying of collagen/enzyme mixture, w or w/o cross-linking w/ glut. (2) electrocodeposition of collagen/enzyme mixture (3) impregnation of enzyme into collagen membrane, w or w/o cross-linking w/ glut.	to examine collagen as enzyme carrier	Activity decreased 40% early then stable over 5 mos. intermittent use and 2 years cold storage. Enzyme absorption follows Langmuir isotherm. Opt pH for immob. is 7.8, between zpc of enzyme and collagen. K_m about 50 times free enzyme - diffusional restriction of large substrate Enzymes w/high MW substrates are generally not good candidates for immob. on solids. Better results are probably obtainable via microencapsulation or semi-permeable membranes (UF).	19

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
3.2.1.22	α -Galactosidase (Melibiase)	Hydrolysis of terminal non-reducing α -D-galactose residues in/dimethyl α -D-galactosides	(1) Porous polyethylene disc coated w/nylon from HCOOH and then enzyme added cross-link. (2) Enzyme added to nylon floc precipitated from HCOOH and cross-linked with glut or DNA.	To examine immob. method.	Enzyme produced from <i>B. stearothermophilus</i> . Yield of enzyme = 95%. 10 mg enzyme/gram nylon. In larger systems, need spacers to hold column. continuous use for 1 mo. w/no change pH opt. = 7 $K_m = 1.4 \times 10^{-2} M$ - same as free. Plugging occurs due to insolubles if molasses or soy milk is added w/o prefiltration and dilution. High bacterial growth at room temperature controlled by 370 ppm HCHO. Plugging controlled by intermittent washes. Nylon support is good for reactions under harsh conditions - however most harsh conditions would probably denature enzyme anyway.	52

I. J. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
3.2.1.23	β -Galactosidase (Lactase) β -D-Galactoside galactohydrolase	Hydrolysis of terminal non-reducing β -D-galactose residues in β -D-galactosides Lactose + glucose + galactose)	Physical adsorption onto phenol formaldehyde resin followed (if desired) by cross-linking w/glut. Also used. Substituted or treated resin w/ or w/o glut. Immobilized on porous silica after silanization by cross-linking w/glut.	For column hydrolysis of lactose in milk whey. To examine enzyme stability in commercial set-up as function of feed composition and to determine operating strategy for hydrolysis of whey.	Cross-linked enzyme retained ~75% activity. Stability increased by cross-linking (no desorption?) w/o glut $\downarrow$ 10% in 7 days, no change w/glut. Loss in activity after 55-60°C for 1 hour. Run @ 450 4 weeks - no problem. Cannot store dry, but 6 mos. cold and wet is fine. Wide variety of resins and resin treatment may work better for different enzymes. Good activity, stability Enzyme (immobilized) inhibited by galactose. $K_m = 0.0528M$, $54 K_i = 0.0054M$. Opt. pH ~ 3.5 $\Delta E_a = 12$ kcal/mole $\Delta E_{deact} = 40.6$ kcal/g mole. To feed whey in, must use UF, deashing and hydrolysis to prevent protein from coating particles w/loss of activity. Several temperature and flow profiles discussed to maximize productivity. After experimentation and theoretical simulations, best strategy found to be isothermal operation of parallel reactors at minimum temperature until conversion starts to decrease, then increase temperature to maintain constant conversion to maximum operating temperature and remove from service. Fairly realistic engineering study of problems in immobilized enzyme system.	53

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
3.2.1.23	B-Galactosidase		Immob. on chitosan (partially deacylated chitin) by cross-linking chitosan w/glut and adding enzyme solution. Entrapment in chitosan gel produced by cross-linking solution of enzyme and chitosan w/various agents Covalent bonding to cellulose via chloro-triazinyl derivatives of cellulose and DEAF-cellulose	To demonstrate advantages of immob. To explain immob. method To examine immob. method	Immob. enzyme operates in several systems (lactose solutions, whey, ultrafiltrates). pH opt. ~3.5. Reduction of particles w/enzyme by NaBH₄ increased stability w/time and T up to 60°C. Stable on storage (2 mos. @ 40 w/3% ↓ in activity) and w/1 month use (80% initial activity retained). Granular gel formed - activity not given in useful terms. Sheets stable for several months. Yield and activity not given.	55 56 5 68

I.C.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
3.2.1.23	Lactase		On porous SiO ₂ + TiO ₂ (~30/45 mesh, 370 Å pores) using silane and glut.	Engineering study of lactose hydrolysis from whey.	Lactose + whey passed through 1.5 cm packed columns. Larger columns (to 4" diam.) also run. Operation @ 90° pH 3.0. Data for column agree w/predictions based on M-M kinetics w/product inhibition. Immob. changed pH opt ↓ 1.0 units, little or no mass transfer limitation. SiO₂ more efficient in coupling enzyme - important if enzyme is expensive. t_{1/2} for system ~ 90 days - better for lactose than for whey. Filtration and salt removal ↑ t_{1/2}. Backflush of columns w/ H₂O restricts microbial growth. Scale-up: using changes in T to maintain conversion, operating cost ~ 1-4 c/lb lactose for 10000 lb/day @ 50% conversion. Use electrodialysis or ion exchange to remove salts - another 3c/lb. Total of 8-10c/lb. (including enrichment of product to sweetener levels) is favorable compared to current sweetener costs. Capital cost (1975) ~ \$100,000. Comprehensive pilot study of lactose hydrolysis.	57

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
3.2.1.23	B-Galactosidase		Entrapment in radiation polymerized gels of: (1)acrylamide (2)dimethylacrylamide (3)2-hydroxyethylacrylate (4)Na acrylate (5)N-vinylpyrrolidone (6)polyvinylalcohol Polyacrylamide gel entrapment in emulsion beads	To examine Immob. method. To characterize immob. enzyme system	Glucose measured by Glucostat. In (1), gelation w/o leaks at 1MMR. 33% act. retained. In (2), 56% @ 2MMR w/o leaks. In (3), gelation at 0.25 MMR, leakage up to 5-6 MMR. In (4), no gel. In (5), gel at 1 MMR, leaks up to 2.9 MMR. Act = 8.6% Acrylamide is best gel for this methodology which seems relatively expensive. 3 types enzyme: from Aspergillus oryzae, E. coli K12. Salactis. Glucose monitored w/glucose oxidase-catalase-chromogen system. Diffusional control of reaction negligible. Low conc. of bisacrylamide (cross-linking agent) increases initial activity but gives excessive leakage. Opt. conc. 2.5%. Some anti-oxidant's increased activity. Opt. pH 7. Same as free enzyme. Immob. enzyme used in batch and plug flow reactors - follows theoretical kinetics for conversion ~ 80% (model includes inhibition by galactose). In plug flow @ 300, pH 6.5, act. decrease 16% after 48 hours. Preliminary study indicates possibilities. Not very good stability.	48
						58

I.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
3.2.1.23	Lactase		(1) casting and drying of collagen enzyme mixture, w/ or w/o cross-linking w/ glut. (2) electrodeposition of collagen enzyme mixture (3) impregnation of enzyme into collagen membrane, w/ or w/o cross-link w/ glut. (1) covalently bound to aminoalkylated porous glass (Corning-80-120 mesh, 2050 Å pores) using glut. (2) cross-linked to ground collagen with glut. (1) precipitation w/ tannic acid, mixing w/ Celite, and cross-link w/ glut (2) cross-linked w/ glut. on phenol formaldehyde (Duolite) resin.	To examine collagen as enzyme carrier To evaluate use of immob. enzyme for lactose hydrolysis in milk processing To examine use of system for lactose hydrolysis	Activity measured by measuring glucose w/ glucostat. 30% of initial activity stably (4 mos.) retained after 4 runs in impregnated membrane. Cross-linking increases retention and activity. Activity, absorption show plateaus with respect to enzyme conc. Temperature, pH profiles very similar to free enzyme. K_m - 0.1M compared w/ 0.07M for free enzyme. Simple first-order analysis indicates effectiveness=0.47 spatially-wound membrane reactor - not bad. Collagen would seem to be a fairly cheap, flexible method for immobilization of many enzymes - initial leakage may be bad if glut. cannot be used. Enzyme taken from <i>S. lactis</i> because pH opt. ~ 7.0. Other enzyme have optimum near 4, less useful for milk. ~ 14 mg enzyme/gram support. pH optimum ~ 7.0 → 6.4. K_m ~ 0.016 to 0.019 (glass), 0.022 (collagen). V_m was 1.66 soluble, 1.60 glass, 1.92 collagen. All activity retained after 120 days @ pH 7.0 (room temperature). Bacterial growth occurred in all cases. Bactericides tended to inactivate enzyme. Bacterial growth is major system problem. Celite used in (1) because precipitate plugs column @ 0.6 ml/min of 3% lactose @ pH 4.5, 450, 90% hydrolysis obtained with immob. enzyme (1). With Duolite, activity opt. ~ pH 4.0; still active @ 600 but w/ loss of activity over time. At 450 for 4 wks, no loss. Cold storage for 6 mos. ok. % hydrolysis is flow limited. Scale-up 500 times ok. Major problem - bacterial contamination and plugging	19 59 60

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
3.2.1.23	β-galactosidase		covalently coupled to polymethylene polyphenylisocyanate molded onto a carrier (magnetic stir bar)	To study applicability of enzyme for hydrolysis of lactose in dairy processing.	Very stable over 85 days use. 95% activity retained over 97 days @ 40° pH opt. increases from 6.5 to 7.5. Good kinetics, K_m ↑ from 13.1 to 21.0. Good kinetics indicates that preparation might be suitable for analytical purposes.	61
			On porous glass - details not given.	To examine optimal conditions for use of glass/enzyme in packed column for whey hydrolysis	Must avoid or minimize: (1) column plugging by protein, especially if denatured by high temperature used to minimize microbial growth. (2) Low conversion if low temperature used to stop germs (3) General minimization of introduction of microbes (4) Proteases in whey cleaving enzyme, or other inhibitors. pH opt. seen @ low pH, but mold still blocked column after 65 hrs. High temperature inactivates enzyme, low temperature gives low rates - both no good. Pretreatment with N_2 or quaternary NH_4Cl minimized growth @ pH 3.5, 40°. Also had problems w/galactose inhibition. Illustrates problems in industrial use of immob. enzyme	62
			Adsorption and cross-linking (cyanuric acid) on Duolite A-7	To examine industrial feasibility of this enzyme immob.	Good stability over 8 days pH 6.5, 25°. Good prospects for industrial reaction.	3
			Bonded to NiO , stainless steel and alumina - method not given.	To use in fluidized bed reactors.	Fluidized bed reactors do not plug easily. Can be disinfected w/no loss in activity, Lower ΔP , good conversion @ large bed expansions. This may be a good idea if microbial growth and/or particulate plugging are anticipated.	63

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I.C.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Res. ts and Comments	Ref(s)
3.2.1.26	Invertase	Hydrolysis of terminal non-reducing β -D-fructofuranosides (including sucrose + glucose + fructose)	Entrapment in radiation polymerized gels of: (1) acrylamide (2) dimethylacrylamide (3) 2-hydroxyethyl acrylate (4) Na acrylate (5) N-vinyl pyrrolidone (6) polyvinylalcohol	To examine immob. method	Glucose measured by glucostat. In (1) gelation w/o leaks at 1 MVRad. Act. = 25%. In (2), 28% at 2MVRad. In (3), gelation at 0.25MM, but leaks up to 5-6MMR. Act = 9.9%. In (4), no gel. In (5), gelation at 1MMR, but leaks to 2.9MMR. Act = 1% - depends on enzyme. In (6), gelation at 4MMR, leakage to 5MMR, Act = 18%. Act = 44% w/more intense rad. for shorter time. Expensive way to immob. enzyme.	48
			Covalent linking to aminoethyl cellulose and porous glass w/glut. or physical adsorption.	To compare immob. techniques for sucrose hydrolysis	Adsorbed enzyme has lower pH optimum than free enzyme. Specific activity 1/3 - 1/2 that of free enzyme. Washing of adsorbed enzyme with 1M or 2.5M NaCl causes nearly complete desorption. In a CSTR, at pH 2.5 desorption occurs for first 4-6 hrs. At 2.0M sucrose, 83% desorption occurs in 6 hrs. Using process sucrose (rather than reagent grade), desorption is more complete. On the other hand, covalently bound enzyme loses no activity over 17 hours. Clear indication of problems w/simple adsorption.	64
			Entrapped within cellulose triacetate	To examine enzyme stability	Very stable. Loss of activity from fibers less than 6% over 48 days. Operationally stable 1800 days. Some mold growth observed.	65

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
3.4.21.1	α -Chymotrypsin	Proteinase: cleavage at Tyr, Trp, Phe, Leu residue	Enzyme was combined with dye-inhibitor and entrapped in polyacrylamide gel.	To develop an immob. -enzyme photographic process.	Process involves the activation of enzyme by UV breakdown of inhibitor. The enzyme then hydrolyzes a second enzyme, pre-tyrosinase, forming tyrosinase, which in turn breaks down D,L-dopamine to melanin, a pigment. All of these were co-immob. onto photographic paper by coating w/a carboxymethyl cellulose. Immobilization of enzyme in thin layers has improved response time down to $\sqrt{15}$ sec. Sensitivity has increased over a direct system (i.e. activated enzyme directly produces dye) because each activated enzyme molecule produces several dye-producing enzyme molecules. Still less sensitive than Ag, and only to UV light. However, may have applications in photo-etching, etc. Sheets stable several months. Yield, activity not given.	134
		Covalent bonding to cellulose via chloro-triazinyl derivatives of cellulose and DEAE cellulose	Contained behind UF membranes while attached to soluble high MW support (dextran, DEAE-cellulose) via triazines.	To examine immob. method.	Chymotrypsin attached to dextran much more active than that attached to cellulose. Stability is also increased relative to free enzyme. Cell fitted with 100,000 MW cutoff membrane was operated using casein as substrate for 2 weeks @ 20°C with full activity. Process needs both immob. and confinement - too complex. Enzyme immob. is 1-2% of total support. Activity ~ 25% of free enzyme, retained up to 60% for 2-2½ years @ 20°C.	5 74
		Covalently coupled to chloro-S-triazinyl derivatives of cellulose		To examine immob. method		66

I.C.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
3.4.21.1	Chymotrypsinogen/Chymotrypsin		attachment to glass via enzyme amino groups to activated glass surface. Covalent coupling to methylimido-esters of polyacrylonitrile @ pH 8.5-10 room temperature, 0.5-5 hrs. Covalently bonded to Sepharose gel activated with benzoquinone	To examine effects of microenvironment on structure, function. To test immob. method To examine immob. method	75% of original activity returned after denaturation. Increased activity toward less specific substrates, indicating conformational change rather than diffusional or electrostatic. Fluorescence spectra similar in native and reoxidized enzyme. Assay via spectro. or titration of ester hydrolysis. Loading of enzyme ↑ w/pH, time. Increased activity and heat-stability compared to free enzyme. At high temperature, stability drop is large then decreases due to variation in enzyme conformation. No enzyme leakage, stable through lyophilization. Activity checked w/N-acetyl-L-tyrosine ethyl ester. 100 mg enzyme/gram gel bound. 78% activity retained. pH optimum shifted from 7.7 to 9.8	7 68 46

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
3.4.21.4	Trypsin	Protease; cleavage at Arg, Lys	Covalent coupling to porous glass by aminoalkylsilane Physical adsorption	To examine immob. method To examine variables affecting adsorption rates. To examine immob. method.	Activity assayed with benzoylarginine ethyl ester (BAEE) spectrophotometrically. 100% initial activity maintained 158 hrs. @ 23°C, 50% at 270 hrs., 16% at 347 hrs. Some increase in stability at higher temperature. Assay using BAEE. Rate and amount of adsorption ↑ with protein concentration and temperature, and with ↓ ionic strength. Adsorption goes through maximum at pH ~ 6. After 72 hrs. adsorption and desorption; all activity is lost by changes in enzyme conformation. Adsorption on glass varied strongly from batch to batch. On quartz pH optimum was more alkaline (7-9). Adsorption not good immob. method. pH activity profile shifted ~ 2-3 units more alkaline. Temperature stability slightly higher. Stored 2 mos. @ 40 w/100% activity retained. Could be freeze-dried and still retain 70% activity after 2 months. About 60% of free enzyme actively immob. Good general method for immob. onto nylon, a cheap, sturdy carrier.	64
			Covalent coupling to substituted nylons Adsorption onto colloidal silica followed by cross-linking w/glut.	To examine immob. method.	Activity determined by spectrophotometer and by activity on benzoylarginine ethylester (BAEE). Adsorption is independent of pH between 6.8-8.7, but decreases above 8.7. Active and inactive protein are equally adsorbed. EM and adsorption equilibrium indicate protein monolayer is formed. 5 times excess of glut. is optimal for cross-linking. Decrease in activity of about 20% occurs on immob., greater for larger substrates - more steric hindrance.	70
					pH activity profile shifted ~ 2-3 units more alkaline. Temperature stability slightly higher. Stored 2 mos. @ 40 w/100% activity retained. Could be freeze-dried and still retain 70% activity after 2 months. About 60% of free enzyme actively immob. Good general method for immob. onto nylon, a cheap, sturdy carrier.	71
					Activity determined by spectrophotometer and by activity on benzoylarginine ethylester (BAEE). Adsorption is independent of pH between 6.8-8.7, but decreases above 8.7. Active and inactive protein are equally adsorbed. EM and adsorption equilibrium indicate protein monolayer is formed. 5 times excess of glut. is optimal for cross-linking. Decrease in activity of about 20% occurs on immob., greater for larger substrates - more steric hindrance.	72

I.C.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Res.-ts and Comments	Ref(s)
3.4.21.4	Trypsin		Covalent coupling to polyacrylonitrile-imidoester methyl ester @ pH 8.5-10; room temp., 0.5-5 hrs.	To test immob. method.	Assay via spectro. or titration using esters. Loading of enzyme $\uparrow$ with pH, time. Typical 1.5-8 mg enzyme/gram. Slightly increased activity of immob. enzyme relative to free. (15-20%). Immob. enzyme also more heat stable than free. Drop in high temperature stability is large initially, then slows, due to variable position of enzyme-polymer linkages maintaining different conformation. $\sim$ stable over 1 year @ 50C. No enzyme leakage, stable through lyophilization. Appears to be a good support-possibly a bit complex for industrial purposes.	68
		Adsorbed and covalently bound (CNBr) to Sephadex		To study effects of microenvironment on immob. enzyme	Some stabilization of the enzyme found due to cross-linking of enzyme structure with polymer. No experimental data given.	73
		Trypsin or succinyltrypsin were covalently attached to derivatized nylon		To retain stability and low cost of nylon and increase binding capacity of nylon for enzymes.	Retention of enzyme activity $\sim$ 80% - Storable for 2 mos. @ 40°. 80% activity retained after lyophilization. pH optimum more alkaline. No figures given for untreated nylon.	74
		Immob. using glut. (details not given) on glass probe		To test concept of thermal enzyme probe (heat generated by reaction causing ΔT activating thermistor)	About 10-20 μ g enzyme bound/probe. Given steady state operation and M-M kinetics at low substrate conc., probe will be diffusion limited, with $\Delta T = DAHS/K_m$, where D is diffusivity, ΔH is heat of reaction, S is substrate conc. Typical values give $\Delta T \sim 10-40C$. Transient responses to changes in substrate are more easily seen.	75
					Delicate instrumentation needed to get reproducible ΔT , but is universally acceptable method. Best with high ΔH reactions.	

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
3.4.22.2	Papain	Proteinase Arg, Lys, Phe-X residues	Covalent coupling to porous glass by aminoalkyl silane Covalent coupling to substituted nylons Physical adsorption on collodion membrane followed by cross-linking w/bisdiazobenzidine 3,3'-disulfonic acid. Physical adsorption on collodion membranes followed by cross-linking w/bisdiazobenzidine - 2,2-disulfonic acid.	To examine immob. method. To examine immob. method To examine properties of immob. enzyme system. To examine properties of immob. enzyme system.	Activity assayed w/casein substrate by spectrophotometer. Very good thermal stability (no change @ 88°C over 80 minutes). After 120 minutes, activity ↓ 60%. pH-activity profile same as free enzyme. Temperature stability decreased. About 30-40% of free enzyme actively immobilized. Good general method for immobilizing onto nylon, a cheap, sturdy carrier. Activity examined on benzoylarginine amide and ethyl ester (BAA, BAEE). Stability good in presence of substrate or after 4 mos. @ 4°C in water. Activity-pH curve for BAA is similar to free enzyme. However, for BAEE activity keeps on increasing with pH - apparently, local pH is lowered by release of products of ester hydrolysis. Shows that local enzyme environment can be very different and thereby change activity profile. Theor: Simple model of adsorption process appears to fit data fairly well. Membrane pore radius found ~ 280 Å compared to 300 Å for membrane w/o enzyme. Expt: pH activity profiles varied with substrate and are different from free enzyme. This is apparently due to local pH changes, especially when ester hydrolysis occurs.	69 71 76 77

I.C.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
3.4.23.1	Pepsin	Protease: Phe, Leu residue	Covalent coupling to substituted nylons	To examine immob. method	pH-activity profile same as free enzyme. Temperature stability same as free. Retained 40% of activity after 2 mos. @ 40C. Lost 92% of activity on freeze-drying. Only 6% of free enzyme actively immob. - procedure needs pH ~ 5 - too high for this enzyme. Good general method for immobilizing onto nylon, a cheap, sturdy carrier. Loaded in packed column. No activity leaked from glass. Activity dropped as pH > 6.2. Stable for several months @ 40C, (30 min, inactivated @ 250. Treatment w/0.05% H₂O₂ (30 min, 150) sterilized w/o affecting activity. Column had tendency to plug, apparently fouled by glycoproteins. Slow loss of activity seen w/continuous operation - reactivation w/.005 M HCl caused faster ↓ in activity but 2M urea (pH 3.5, 60 min) was good reactivator.	71 29

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
3.5.1.1	L-Asparaginase	Asn + H ₂ O + Asp + NH ₃	Covalent binding to alkylamine glass or aminoethyl cellulose using fluoronitroazidobenzene a photo activated reagent.	To examine immob. method and enzyme properties	Storage dry at room temperature retains 90% activity for 1 month. 86% activity retained for 7 days wet at 37°, 97% for 21 days at 48°C. pH activity curve is sharper, slightly more alkaline than for free enzyme. Temperature activity curve is similar. Very mild reaction conditions result in good activity - may be good for fragile enzymes.	18
			(1) casting and drying of collagen enzyme mixture w/ or w/o cross-linking w/ glut. (2) electrocodposition of collagen enzyme mixture (3) impregnation of enzyme into collagen membrane, w/ or w/o cross-link w/ glut.	To examine collagen as enzyme carrier.	Activity measured by NH ₃ production. Activity decreased to stable level ~ 50% of initial after 2 runs, retained over 4 mos. pH/activity about the same as free enzyme. Km ↑ 80 times - some steric blockage. Continuous flow-through reactor operated stably for 5 days. 1 ml/min of 4mM Asn, 37°, pH 8.5, 19% conversion. No difficulties here - electrostatic effects thought to have produced high Km	19
			Added to collagen dispersion, cast, dried and tanned (cross-linked) w/glut.	To examine system for removal of asparagine from blood	Collagen sheets wound onto polymer spaces in spiral. Stable after 4 mos. @ 40°. Km ↑ 80 times. Activity vs pH about same as for free enzyme. Authors feel this might be better than cells, microcapsules, etc. However, this requires extracorporeal blood treatment - not so good.	79
			Immob. on sand-blasted γ -aminopropyltriethoxysilane treated Lucite plates. Silane then reacted w/glut. Then add enzyme.	To use enzyme to decrease asparagine levels in blood as an anti-tumor technique	Asparagine (4x10 ⁻³ to 4x10 ⁻⁴ M) added to blood and passed through parallel enzyme bound lucite plates @ 15 ml/min. Enzyme stability ↑ by immob. from 60-70 hrs to > 300 hrs (tr). Very little enzyme leaching (< 0.2%). Kinetics changed (V _m ↑ 50%, Km ↑ 50 times) - probably diffusional problems. This is still good enough to reduce Asn conc. by 10 times in human trials, but levels return in 3 hrs. The short-term efficiency suggests that extracorporeal treatment will not be good. Artificial cells may be better.	80

I.-B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Res ts and Comments	Ref(s)
3.5.1.5	Urease	NH_2CONH_2 $\xrightarrow[\text{CO}_2]{\text{H}_2\text{O}}$ 2NH_3 +	Entrapment of enzyme into a swollen collagen membrane	To examine the stability of a urease system as an aid in hemodialysis	Membrane layered on a cellulose acetate membrane which is spirally wound in a reactor with transverse flow. % activity decreases to 25% of start after 14 12-minute runs w/wash in between - due to leaching out of enzyme. Claim that scale-up could be effective in hemodialysis with NH_4 included. Not a very effective dialysis scheme - needs two stages to remove urea. Blood contacting will also be a problem.	81
			Covalently bonded to partially hydrolyzed nylon tubes with glut.	To examine immob. method.	Activity determined by measurement of ammonia concentration. Protein bound - 62.5 μg /meter of tubing (0.1 cm ID) K_m same as free enzyme. pH activity profile same as free enzyme, also temperature - activity profile. Temperature stability is increased by immob. Preparation of enzyme attached to tubes useful in automated analyses.	82
			Microencapsulation in hydrocarbon based liquid surfactant membranes	To examine immob. method	Enzyme assayed by titration w/urea or by spectrophotometry using indicator. No detectable leakage of enzyme out of microcapsules. $K_m = 0.18\text{M}$, about 50 times free enzyme. Early work on liquid membranes. Very high K_m relative to other immob. methods, but technique is much milder.	83
			Entrapment in polyacrylamide gel on dacron or nylon nets	To develop a sensor for urea	Net placed over cation-selective electrode responsive to NH_4^+ V a conc. up to 30 mg/100 ml. Response time ~ 100 sec. Can measure as low as 10^{-4}M . Maximum response obtained @ ~20 mg enzyme/cc gel. Gel composition not critical. Gel thickness does not affect SS response. Electrode also responds to Na, K, Li; Ag inactivates enzyme. To get good selectivity: $[\text{Na}] < \frac{1}{2}[\text{urea}]$ and $[\text{K}] < 1/5 [\text{urea}]$ is required. At high $[\text{urea}]$, response is independent of conc. - maximum measurable is $1 \times 10^{-3}\text{M}$. Stability $\downarrow$ with $\uparrow [\text{urea}]$ decreased gel thickness, decreased $[\text{enzyme}]$. Covering w/ cellophane decreases enzyme leakage from gel.	84

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
3.5.1.5	Urease		Electrocodeposition w/collagen in rectangular cell w/anodes at each side and cathode control. Current $\sim 4\text{mA/cm}^2$ 50°C, pH 3.8-4.5 or 10.4 collagen conc 0.45%.	To examine immob. enzyme method.	Limits on K, Na limit usefulness in biological solutions (need Tris buffer). Collagen deposits on cathode pH 2.5-5.3, anode 9-12. Activity $\sim 51\%$ pH profile same as free enzyme. Salts in enzyme solution must be dialyzed out first to get deposition	24 25
			Cross-linked w/glut. on aminoethyl cellulose	To study enzyme kinetics in packed bed reactors	Sigmoid plots of rate vs [urea], especially at low [urea]. Classical M-M kinetics would show linear increase in rate with [urea] - this is experimental. Suggest diffusion is problem. Immob. gives different diffusional problems than free enzyme.	23
			Entrapment in gel (polyacrylamide?)	To study kinetics of a urea enzyme electrode to determine optimal physical-geometric parameters.	Model uses one-dimensional equation for diffusional transport of urea into enzyme layer and production (Michaelis-Menten kinetics) of NH_4^+ and transport to Nernstian electrode through porous glass. Increased enzyme loading increases signal and then reaches a plateau due to complete conversion of urea. This implies that an excess of enzyme should be used. At high loading of enzyme, membrane thickness can be estimated from activity - use 2-3 times this amount of enzyme to allow for enzyme inactivation. Fairly straight forward relatively simple scheme.	24

I...5. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
3.5.1.5	Urease		Covalently bound to Enzygel (Boehringer) or Enzacyl AA (Aldrich)-method not given.	To develop specific enzyme electrode for urea.	pH electrode used to monitor change in pH due to aqueous ammonia release by hydrolysis of urea. Response time increases with urea concentration since process is non-linear and requires recalibration. Response times range from 2-4 minutes. Urea can be detected as low as 10 ⁻⁵ M under ideal conditions and 10 ⁻⁴ M normally. No interference from Na ⁺ , K ⁺ , NH ₄ ⁺ seen. Stability over 4 weeks excellent, although response time increases with age. Primary advantage is that since electrode measures gaseous ammonia released from solution, it does not contact solution at all. Slow response time but fairly specific electrode - adaptable to other species that can be found in gas form (CO ₂ , SO ₂).	85
			Covalently coupled to glass silanized with 8-aminopropyltriethoxysilane after diazotization.	To examine characteristics of enzyme system.	Activity assayed via NH ₄ ⁺ -sensitive electrode. Activity constant over 30 days. Maximum activity reached at [urea] ≈ 0.17M. Substrate inhibition at ~ 0.34M. pH optimum at 6.0 - for free enzyme 6.4-7.6.	86

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
3.5.1.11	Penicillin Acylase	benzylpenicillin + 6-amino-penicillanic acid	Covalently coupled to carboxymethyl-cellulose (CMC) or Amberlite (phenolic) resin	To examine immob. enzyme kinetics	Assayed via titration of acid with NaOH. Bromthymol blue was also adsorbed to Amberlite as indication of local pH changes. Coupling to both supports was best at low ionic strength and near protein isoelectric point (pH 6.2-6.7). Immobilization begins at outside of bead and penetrates interior increasingly with time. K_m 50 apparently increased for both supports by up to 50 times. Addition of buffer increases activity - indicator shows that unbuffered beads were strongly acid, while buffered beads had neutral surface, acid interior. This shows importance of local effects in immob. enzyme kinetics. Good study showing microenvironmental effects on kinetics.	87

I...B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
3.5.1.14	Amino- acylase	Acyl-L- amino acid H ₂ O acylate + L-amino acid	(1) Fixed to carrier (porous clay) by unknown method. (2) Trapped behind membrane. (1) Behind ultra- filtration membranes (2) On carrier (method not given) Enzyme placed in recirculating loop w/substrate. Product and "un- reacted sub- strate diffuse through UF membrane.	To compare economics for two systems making L-Met. To compare differ- ent reaction systems and to perform kinetic studies. Production of L-amino acid from DL-mixtures by acylating mixture deacylating L form separating and recycling D to racemizer.	Model of kinetics and all costs optimized by direct search. Membrane reactor superior to plug flow reactor up to 85% conversion and to a two-reactor series up to 91%. (Not clear if this is optimal or not.) Relatively high cost of carrier - immobilization can be difference in choice of reactor. Difficult to understand "English" used. Conversion measured by polarimetry. pH opt. = 7.0. Other results not at all clear (bad writing.) Reaction monitored w/polarimeter. Polarimeter signal can be used to operate pump to add additional enzyme and maintain activity. Must maintain turbulent flow to avoid concentration polarization at membrane surface. This system, using additional enzyme allows the deactivation of enzyme to be measured. t _{1/2} by this method is usually shorter than that measured by substrate conversion, since the change in conversion is not equal to the change in activity. Fairly simple method for immob. - only good with small substrates and reliable, sturdy membranes w/good control of permeability.	98 89 22

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
3.5.1.14	Amino-acylase		Covalently bound to DEAE-Sephadex also tried acrylamide entrapment and covalent bonding to iodoacetyl-cellulose.	To separate DL-amino acids by acylation of DL, deacylation of L only, then separate by solubility and recycle D form after racemization	Loaded into packed column. Cost ~ 60% of batch method w/soluble enzyme. Regeneration of enzyme is possible. $t_{1/2}$ ~ 65 days at 50°C. First industrial use of immob. enzyme.	90
			Adsorption and cross-linking with cyanuric acid on Duolite A-7. (Phenolformaldehyde resin)	To test this immob. method in industrial application	41% of activity retained. Good stability over 10 days at pH 8, 37°C. Good prospects for industrial work.	86 3

I.C.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
3.5.2.6	Penicillinase	Penicillin + H ₂ O → penicilloate	Covalently coupled to glut. activated nylon tube (12.5% Glut. in 1.1M borate buffer pH 9.0 10 min, 25°C; then enzyme in 0.1M N-ethylmorpholine buffer, pH 8.0 for 3 hrs. at 10)	To use system for automated determination of penicillin	Conc. determined colorimetrically by measuring change in indicator due to production of penicilloic acid by enzyme. Can measure 20-100 µM. Over 14 days (12,000 measurements) activity was stable. Also monitored penicillin production in fermentation process for 50 hr. w/o difficulty.	1

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
3.12.1.1	Parathion Hydrolase	Parathion (0,0-diethyl-O-nitro-phenyl phosphoro-thioate) + products	Bound to porous glass by azide coupling	To examine use of enzyme in removing pesticides from aqueous streams.	System tested as fluidized-bed reactor containing 200u of enzyme. Enzyme extracted from cells adapted to growth on parathion. Optimum temperature 35°C, deactivation at 50°C. Optimal pH 9.5-9.5. Organic solvents inhibit reaction significantly, but effects of salts and wastewater are low. With inlet conc. of pesticide at 10 ppm greater than 95% of pesticide was removed. Enzyme stable for 40 days in wastewater. Six weeks stable operation estimated necessary for cost-effectiveness. Whole cells might be superior. Preparation studies in batch, small test columns, and fluidized bed. Increased amount of enzyme bound/wt. carrier reduced activity/wt of enzyme (diffusional resistance). Optimal pH between 8.5 and 9.5, same as for free enzyme. Optimal temperature 35°C, stable at 50-55°C for 15 min. Fluidized-bed reactor had less difficulty handling salts in wastewater and equal efficiency to fixed-bed. In small fixed-bed or batch reactor, activity stayed constant for 70 days of continuous use, and, after initial drop, over 180 days of batch use (15 runs). Various organic solvents reversibly inhibit enzyme. Whole cell preparation might be more resistant to inhibition and cheaper to prepare.	43
			Covalently coupled by azide method to controlled pore glass and silica beads.	To examine use of enzyme in treatment of pesticide-containing wastewaters.	Activity assayed by measuring p-nitrophenol. Apparent K_m of reaction increased by 52% on immob. probably due to diffusional resistance. Temperature and pH profiles were very similar for free and bound enzyme (opt: 8.5-9.5, 35°C). Stability appears good-much better (280 days half-life) under continuous use than under intermittent use (38 days). Author also mentions other enzymes found that hydrolyze several other classes of insecticides.	129
			Covalent coupling to ground glass via azide method	To examine use of enzyme to detoxify pesticide-containing wastewaters.		88
						130

I.C.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
4.1.2.11	D-Hydroxynitrile Lyase	Mandelonitrile = HCN + benzaldehyde	(1) Entrapment in polyacrylamide; (2) Covalent attachment to polyacrylamide-maleic acid copolymer; (3) Copolymerization with acrylamide of alkylated enzyme	To compare effects of immob. method.	Copolymerized enzyme - 13 U/g; Trapped - 2.3; Crosslinked - 1.3. Good stability even in non-aqueous solutions. Demonstrates advantages, at least in this case, of protein copolymerization. Disadvantage is increased complexity of immob. method. Advantages: no gel swelling or shrinking, no adsorption by gel, good kinetics and activity.	12

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
4.1.2.13	Aldolase	D-Fructose-1,6 bis-phosphate → dihydroxy-acetone phosphate + D-glycer-aldehyde-3-phosphate Cofactor: Zn	Covalent coupling to poly (4-methacryloxybenzoic acid) via N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline	To examine immob. method	Good immobilization. No properties give.	35

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IMMOBILIZED ENZYMES/BACTERIA FOR NAVAL APPLICATIONS - INITIAL D--ETC

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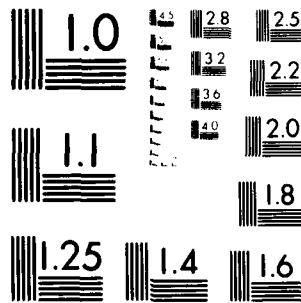
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I. B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Res. ts and Comments	Ref(s)
4.1.99.1	Tryptophanase	L-Trp + H ₂ O → indole + pyruvate + NH ₃ Cofactors: Pyridoxal phosphate (B ₆)	Immob. to (1)CNBr activated sepharose (2)Sephacrose derivative w/alkyl side chain (3)Sephacrose-B ₆ derivative, fixed with NaBH ₄	To examine immob. of B ₆ enzymes.	Most active result obtained from B ₆ - Sepharose (81% immob., 60% activity). pH curve shifted ~1.0 units alkaline. Temperature profile ~ same as free. Enzyme lost activity gradually unless more B ₆ was supplied in feed. Over 90% of indole in feed converted in continuous reactor w/fair stability. Also could use enzyme to determine L-Trp Cofactor - requiring enzyme; even w/ immob. of cofactor still seems to be a problem with stability and retention of cofactor.	91

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
4.1.99.2	β -Tyrosinase	L-tyrosine + H ₂ O = phenol + pyruvate + NH ₃ Cofactors: Pyridoxal phosphate (B ₆)	(1) CNBr-activated Sepharose (2) Sepharose derivative with sidearm (3) Sepharose-B ₆ derivative, fixed with NaBH ₄	To examine immob. of B ₆ enzyme	Best immob. using linkage to CNBr activated Sepharose (B ₆ derivatives appear to block active sites). pH opt. shifted + 1 unit Good temperature stability. Activity lost steadily w/o B ₆ addition. Cofactor requiring enzyme have difficulties w/cofactor retention.	91

I. B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Re - ts and Comments	Ref(s)
4.2.1.2	Fumarase	Fumaric acid + H ₂ O → L-malic acid	Enzyme and substrate in recirculating loop behind UF membrane	To produce malic acid	Reaction monitored w/polarimeter. Two different fumarase sources: pig heart and microbial. Pig heart deactivated within 2-3 days, microbial stable > 1 week. Hydrophobic UF membranes stability, as does addition of hydrophobic polymers to solution. Enzymes catalyzing same reaction from different sources can be very different in stability. The microbial enzyme generally more stable.	22

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
4.3.1.1	Aspartase (L-Aspartate ammonia-lyase)	L-aspartate = fumarate + NH ₃	Adsorption	To compare different resins for enzyme immob.	Activity varies from 0 to 9.6 IU/ml resin. (Best is DUOLITE A-20 - phenolformaldehyde)	3

I.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Re Its and Comments	Ref(s)
5.3.1.5	Glucose Isomerase (D-xylose ketolism- er-ase)	Glucose → fructose	Varied	Description of manufacture of high fructose corn syrup as sweetener from corn starches	Production of 2,000 lbs. 42% fructose syrup/lb enzyme/1000 hrs is typically obtained. The 400-500 hrs. Feed > 91% dextrose needed. Typical conditions are: 93-96% dextrose (40-45% solute) pH 8.2-8.5, 60°C, 0.004M Mg ⁺⁺ , residence time 0.5-4 hrs. Total activity maintained with parallel columns. (usually > 6 commercially), with gravity feed (down-flow). Simple M-M kinetics w/ exponential 1/2-life seems to work as model. Bed must be pre-swollen and pressure drop controlled to avoid compaction. Complete discussion of commercial immob. enzyme system major world application.	92
			(1) adsorbed onto Duolite resin (2) coupled to Duolite with triazinyl chloride	To examine suitability of immob. for industrial reaction.	Resin packed into column for studies. Covalently bound enzyme is better retained and more active. Enzyme does not have to be fully purified. pH opt slightly lower and broader. Free and immob. enzyme both T-stable (best was covalently bound enzyme). Good long-term stability. In larger column (2.5 x 42 cm) with pretreat to remove air and impurities get good stability, no growths (because temperature = 60°). Large scale eng. study indicates immob. enzyme costs much lower than batch w/free enzyme.	13

I.U.B. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
5.3.1.5	Glucose Isomerase		Covalent attachment to porous glass (silanization and cross-link w/ glut)	Engineering study of kinetics and mass transfer	Uses recirculating packed column (pseudo-batch conditions) Glucose analysis w/ Beckman Analyzer. Opt. conditions - pH 7.0, 50°C, 10^{-4} M Co^{++}, 10^{-2} M Mg^{++}. Enzyme retains 56% of activity after immob. Batch reactor model based on reversible M-M kinetics agrees well w/ expt. assuming (K_m) app. $\gg (K_m)$ free. Equilibrium const. = 1.03 same as free enzyme. At higher temp., reaction becomes 1st order so plug flow reactor is better than CFSTR. t_d 240 days @ 50°C based on extrapolation of short time data. However, after 30 days, rapid deactivation (probably due to microbial growth) occurred. Under 60°C and at high flow, reaction is limited by flow and by enzyme kinetics, not by diffusion. Packed and fluidized beds have similar kinetics. Bench scale engineering study. This should be done before any industrial scale-up - must be redone for every new immob.	74
		(1) adsorption onto phenol-formaldehyde resin (2) adsorption and cross-linking cyanuric acid to phenol-formaldehyde or polystyrene resin	To examine the industrial suitability of resins for packed-bed immob. enzyme reactors		Glucose isomerase activity ranged from 1.6 - 32.6 IU/ml resin. Best results on phenol-formaldehyde. Fair stability (pH 8.2, 60°, 6 weeks). Acid cross-linking retained 30% of activity. Other enzymes also checked via same method, but less well characterized.	3

I-3. Class Number	Common Name(s)	Reaction Catalyzed/ Cofactors	Immobilization Method	Purpose of Study	Other Methods Re. its and Comments	Ref(s)
5.3.1.5	Glucose Isomerase		Zirconia coated controlled pore glass (20/30 mesh, 350 ± 35Å pores) treated with silane, washed, treated w/glut. (2.5%), washed and reacted w/enzyme.	To examine engineering capabilities of system.	Glass used in well-stirred batch reactor w/glass frit to keep in beads. Compared w/soluble enzyme preparation. Monitored conversion w/polarimeter. Very little change in kinetics for immob. enzyme. Activity decayed exponentially w/time. Flow rate did not affect t_R. Activity increases but $t_R \downarrow$ w/temperature. Mass transfer not rate-controlling step in system. Operation will be isothermal (low heat of reaction). For commercial operation, system will probably be run at constant conversion (decreasing flow with time as activity drops). Design estimates call for 11 columns 6" wide, 36" long run @ 60°C to produce 10⁷ lbs/yr of 50% glucose w/45% conversion. AP ~6.4 psi. Fairly detailed bench-scale examination of engineering of enzyme process. Shows advantages of CFSTR/plug flow over batch processing.	95

Common Name(s)	Immobilization Method	Purpose of Study	Results and Comments	Ref(s)
ANP	Derivatization to N ⁶ (6-aminohexyl) AMP or N ⁶ -(6-aminohexyl)-amino cyclic AMP followed by coupling to CNBr activated Sepharose	To examine "spacer" AMP as immob. coenzyme and as affinity chromatography ligand	Separation of enzymes achievable. Usually need to change column w/enzyme and then add a third substance to get specificity of binding. Major application thought to be as bound cofactor.	14

Common Name(s)	Immobilization Method	Purpose of Study	Results and Comments	Ref(s)
Coenzyme A	CNBr activated Sepharose	Adsorption of enzyme w/affinity for Coenzyme A	Several Coenzyme A using enzymes could be adsorbed with good concentration from crude extracts. The purified extracts could in turn be used to purify crude Coenzyme A extracts. Generally useful but no specificity for any particular enzymes.	97

Common Name(s)	Immobilization Method	Purpose of Study	Results and Comments	Ref(s)
Coenzyme B ₆ (Pyridoxal 5'-Phosphate)	Covalently linked to either p-aminobenzamido-hexyl-Sepharose or bromoacetyl Sepharose	To study activity of immob. B ₆ w/B ₆ requiring enzymes	Activity depended on location of bond to B ₆ bonding at pos. 6 had good activity. At 1 position less activity obtained as no activity if covalent bond was at 3 (-OH) position on pyridine ring.	91

Common Name(s)	Immobilization Method	Purpose of Study	Results and Comments	Ref(s)
NAD	derivatization to NAD-N ⁶ -(N-6 amino hexyl acetamide) or N ⁶ carboxymethyl NAD followed by attachment to CNBr Sepharose	To examine "spacer" NAD as immob. coenzyme and/or affinity chromatography ligand	Efficiency of substituted soluble analog was 50-100%. Activity also obtained in bound state but figures not given. Major application thought to be as bound cofactor.	96
NAD	Physical adsorption on carbon electrodes	To examine possible direct regeneration of NAD	At about 0.7V, selectivity of oxidation and reaction rate yield optimal turnover of NAD. At this level, specificity is 90-95% - much too low for commercial viability (need at least 99.9%) Very long shot possibility due to high specificity needed.	98
NAD	Cross-linked w/albumin and alcohol dehydrogenase using glut.	To show that cofactor can be immob. in active state	NAD regenerated using O ₂ and phenazine methosulfate as electron carrier. When high NAD concentration is used, NAD is immob. within enzyme, resulting in deactivation. Optimum concentration ~ 10 ⁻³ M. Membranes were used on PO ₂ electrode and showed NAD was functional. Limited application of membranes - no data on stability.	2

Common Name(s)	Immobilization Method	Purpose of Study	Results and Comments	Ref(s)
Riboflavin	Covalent attachment to activated carbon	To employ cofactor as an electron transfer agent to extract electrical energy from biochemical reactions.	Some cofactor immobilized, but only in very low concentrations. No experimental work with immob. cofactors.	/ 20

Common Name(s)	Immobilization Method	Purpose of Study	ner Methods Results and Comments	Ref(s)
P. putida ATCC 4539	Polyacrylamide	To examine industrial potential of cells to make citrulline by deimination of arginine	Packed gel into column. 0.5 M Arg in feed @ pH6, 37°C. Conversion ~ 100% for space velocity <0.26. $t_{1/2}$ - 140 days @ 37°C, unstable at 50°. Stability increases with decreasing flow rates. Industrially feasible operation, but application will depend on market factors.	90

Common Name(s)	Immobilization Method	Purpose of Study	Results and Comments	Ref(s)
Bacteria from washed soil sample	Cast suspension of collagen/bacteria on plate and cross-linked with glut.	To construct BOD sensor	Bacterial membrane placed over teflon membrane on O₂ electrode and exposed to wastewater. Current decreases to steady-state level related to BOD of wastewater. Steady state reached in 10-15 minutes at 30°C. Current is weak function of pH, independent over 6.5-8.0, increasing at higher or lower values (decreased activity). Current is sensitive to presence of Cu⁺⁺ ions but not other heavy metals. Current proportional to BOD over 0-22 ppm. Electrode stable for 10 days at 30°C. Results within standard error of standard BOD method, requiring 5 days. Good for low BOD samples (<20 ppm). Fuel cell method shown in same reference useful for higher BOD samples.	/25

Common Name(s)	Immobilization Method	Purpose of Study	Results and Comments	Ref(s)
Achromobacter liquidum IAM 1667	Polyacrylamide entrapment	To make urocanic acid, used as sunscreen, from histidine	Cells have enzyme, urocanase, which degrades acid. This is removed by heat treatment (70°C, 30 min) before immob. & does not affect lyase. Packed cells in column. 0.25 M His (pH 9.0, 1mM Mg⁺⁺) had 100% conversion for space velocity < 0.06. Acid is crystallized easily from output. $\bar{t}_k \sim 180$ days @ 37°.	9C

Common Name(s)	Immobilization Method	Purpose of Study	Results and Comments	Ref(s)
Aspergillus niger	Coproccipitation of collagen particles and cells followed by tanning with glut.	To examine potential of cell immob. system.	Membrane wound into spaced, spiral reactor with counter current oxygenation. Up to 70% cells can be bound, but over 50%, membrane strength $\downarrow$. To get opt. yield, must induce required enzymes in cells, e.g. by batch fermentation and then limit cell growth by limiting nutrients so that metabolism will proceed as desired. This also limits contamination. Good reactors have been made from spirally-wound membrane. Net cell activity $\sim$ 50% of free cells in fermentation (for citric acid production). $t_d \sim$ 138 hrs. General review of collagen-cell reactor based on one organism.	101

Common Name(s)	Immobilization Method	Purpose of Study	Results and Comments	Ref(s)
A. niger	Spores physically adsorbed in glass carriers. Carriers had pore sizes ranging from 0-195 μ .	To examine effect of pore size on cell accumulation.	Biomass assayed by ATP measurements. Spore size 3-5 μ . Maximum mycelial growth at about 40 μ pore size. Useful study on pore size requirements for different microbes, showing effect of reproduction method. Gives general guidelines as which should apply to any type of cell.	102

Common Name(s)	Immobilization Method	Purpose of Study	Results and Comments	Ref(s)
Bacillus	Physical adsorbed onto glass carriers. Carriers had pore sizes ranging from 0-195 μ .	To examine effect of pore size on cell accumulation.	Biomass determined by ATP assay. Cell dimensions 3-4 μ . Maximum accumulation at about 6-8 μ , lower than some other fissioning cells, but in same general area. Useful study on pore size requirements for different microbials, showing effect of reproduction method. Gives general guidelines which should apply to any type of cell.	10-2

Common Name(s)	Immobilization Method	Purpose of Study	Results and Comments	Ref(s)
Chloroplasts/Hydrogenase	Several - covalent binding, entrapment, hollow fibers, adsorption	To examine possible H ₂ production by plant cells or extracts from radiant energy using chloroplasts to reduce ferredoxin with light, and hydrogenase to reoxidize ferredoxin and produce H ₂	All studies done on impure cell extract. Highest activity from immob. by adsorption on RPC-5 (polychlorotrifluoroethylene coated with methyltricapryly ammonium chloride), retains 10% of free system activity. This may be increased by using purified enzyme. Major problem is fast re-oxidation of reduced ferredoxin by O ₂ before reaching hydrogenase improved separation process needed.	143

Common Name(s)	Immobilization Method	Purpose of Study	Results and Comments	Ref(s)
Cl. butyricum	Immob. on nylon net attached to platinum anode by entrapment in polyacrylamide gel	To measure BOD of wastewater	Using carbon cathode with phosphate buffer as catholyte and waste water as anolyte (chambers separated by ion-selective membrane) a fuel cell was set up reducing the H ₂ produced by the immob. cells. High current at start decreased in ~ 30 min to constant level proportional to BOD over 0-300 ppm. Optimum conditions were pH 7, 37°C. Reproducibility was about 7% and standard deviation was about 2 ppm. Error of estimate ~ 10%. Electrode was stable for about 30 days and could be stored at 50°C for 40 days. Fairly rapid BOD estimator. Good reproducibility, since controlled microbial population was used.	125
Cl. butyricum	Immob. on Pt electrode via entrapment in polycarylamide gel.	To construct biochemical fuel cell using immob. bacteria.	Cell has anode and cathode chamber connected by agar salt bridge. Pt/bacteria electrode is anode, anolyte is phosphate buffer with glucose. Cathode is carbon electrode in phosphate buffer. At opt. pH of 7.7, cell potential was -0.65V at current densities up to 2 $\mu\text{A}/\text{cm}^2$. Cell produced 1.1-1.2 mA constantly for 15 days while consuming 2.7 millimoles glucose/day. Questions have been raised as to whether this current was developed via H ₂ produced by cells or directly from a glucose oxidation.	135
Clostridium butyricum	Entrapment in agar or polyacrylamide gel	To produce H ₂ from wastewater for fuel cell.	H ₂ production higher in agar. Cells stable for 20 days. In packed bed, maximum H ₂ at flow rate of 5-10 ml/min. Fuel cell produced 14 mA at 0.63V. using Pt black electrode w/ waste of BOD - 3300 ppm. Final BOD of effluent ~ 45 ppm, also maintained 20 days.	104

Common Name(s)	Immobilization Method	Purpose of Study	her Methods Results and Comments	Ref(s)
E. coli	(1)by adsorption (2)by adsorption and linkage to amino-silanzed carriers. Carriers had pore sizes ranging from 0-19 μ	To examine effect of pore size on cell accumulation	Biomass measured by ATP assay after 18 hours. Maximum growth occurs at about 5 μ for both methods, about 5 times smallest cell dimension - this seems typical for cells growing by fission. (E. coli dimensions = 1-6 μ). Useful study on pore size requirements for different microbes, showing effect of reproduction method. Gives general guidelines which should apply to any type of cell.	102

Common Name(s)	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
E.coli ATCC 9637	polyacrylamide gel	To produce 6-amino penicillinoic acid (6-APA) starting point for synthesis of penicillin analogs by amidation of penicillin.	These cells also have penicillinase activity. At proper conditions, amidase is more active. 0.05M penicillin G. @ pH 8.5 passed through column. 80% yield of 6-APA at space velocities from 0.12-0.24 t_R 42 days (30°C). Free enzyme not stable.	90

Common Name(s)	Immobilization Method	Purpose of Study	ther Methods Results and Comments	Ref(s)
E. coli	(1) entrapment in acrylamide (2) cross-link of cells w/ glut or 2,4-toluene diisocyanate (3) encapsulation by polyurethane. Best was (1)	To make aspartate from fumarate	Loaded into packed column for conversion of fumaric acid by feeding ammonium fumarate to column. Activity increased initially with time due to autolysis of cells in gel and increased permeability to substrate w/o enzyme leakage. Opt. act. at pH 9.5 for immobilization, same as free enzyme while free cell @ 10.5. Opt. temperature = 50°. Ca, Mg, Mn ions stabilize activity. $t_{1/2}$ ~ 120 days @ 37°, decreases with ↑ temperature. Cost ~ 60% of batch system. First industrial use of whole immob. cells.	90
E. coli (ATCC 11303)	Trapping in acrylamide gels	To use whole trapped cells w/ aspartase activity to produce L-aspartate from fumarate	Packed gels into column - looked for conversion of ammonium fumarate to aspartate. Whole cell optimum activity at pH 10.5, 50°C - after immob., opt pH = 8.5. Enzyme above has pH opt. at 9.5, = 37°C. Bivalent metals (Mg^{++} , Na^{++}) activate enzyme, stabilize cells. Packed column deteriorated @ temperatures of 45 and 50°. Stable @ 37°C.	105

Common Name(s)	Immobilization Method	Purpose of study	Other Methods Results and Comments	Ref(s)
Penicillium chrysogenum	Spores physically adsorbed in porous carriers. Carriers had pore sizes ranging from 0-195 μ	To examine effect of pore size on cell accumulation	Bioassay of accumulation by protein content. Organism prefers less negatively charged carriers. Spores 2.5-4.5 μ . Maximum growth in pores $\sim$ 72 μ . Useful study on pore size requirements for different microbes, showing effect of reproduction method. Gives general guidelines which should apply to any type of cell.	102

Common Name(s)	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
Saccharomyces amurcae	Covalently bonded to polyisocyanate treated glass carriers. Carriers had pore sizes ranging from 0-195 μ .	To examine effect of pore size on cell accumulation	Biomass assayed by ATP measurement. Cell dimensions - 25% single cells (3-7 μ x 6-9 μ), 75% double cells (6-8 μ x 13-18 μ). Double distribution is reproduced in curve of biomass vs carrier pore size. Useful study on pore size requirements for different microbes, showing effect of reproduction method. Gives general guidelines which should apply to any type of cell.	102

Common Name(s)	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
Whole cells - Pseudomonas	Cells allowed to grow and attach themselves to 30/60 mesh coal or sand particles	To test fluidized bed bioreactor for denitrification of wastes	Particles added to 5 or 10 cm ID (tapered bottom) glass reactor. Larger diameter better because of bubble formation interfering w/fluidization. Excess biomass generated by growth removed at top of bed by removing particles, washing off bacteria and returning particles to column. Bacterial survival requires No ₃ in feed less than 7% - higher feed concentrations are diluted by recycle of effluent. Opt. temp. ~ 22°C.	104

Common Name(s)	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
<i>Saccharomyces cerevisiae</i>	Physical adsorption onto glass carriers. Carriers had pore sizes ranging from 0-195 μ .	To examine effect of pore size on cell accumulation	Biomass assayed by ATP measurement. Cell dimensions 2.5-4 μ x 4-7 μ . Cells reproduce by budding. Cell dimensions have a biphasic distribution which is replicated in the curve of biomass vs carrier pore size. Useful study on pore study requirements for different microbes, showing effect of reproduction method. Gives general guidelines which should apply to any type of cell.	102
<i>Saccharomyces cerevisiae</i>	Cells adsorbed onto metal linked to polysaccharide particles.	To examine immob. method.	Cell viability estimated via O ₂ uptake. About 50% of cells are immob. with O ₂ uptake measured several times that of free cells - activation by metal? No desorption of cells seen, particles are stable 6 mos. at room temperature. Immob. appears to be fairly durable. Reason for increased O ₂ uptake unclear.	107

Common Name(s)	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
Serratia marcescens	Covalently coupled to polyisocyanate treated glass carriers. Carriers had pore sizes ranging from 0-195 μ .	To examine effect of pore size on cell accumulation	Biomass measured via ATP assay. Cell dimensions 0.6-2 μ . Maximum growth @ about 4 μ , typical of fission reproducing cells. Useful study on pore size requirements for different microbes, showing effect of reproduction method. Gives general guidelines which should apply to any type of cell.	102

Common Name(s)	Immobilization Method	Purpose of Study	Other Methods Results and Comments	Ref(s)
Streptococcus faecalis ATCC 8043	Entrapped in polyacrylamide	To examine micro-organism	Activity assayed by arginine conversion. Electron microscopy of gel performed to examine immob. (1)At low flow, all arginine is converted. (2)Additional ornithine is produced, probably from second pathway, over arginine input. (3)No citrulline is seen in output. Appears to indicate a highly organized, efficient metabolic path. (4)Use of frozen cell paste leads to high enzyme leakage. Shows clear advantage of whole cells in multistep procedures, especially when cofactors involved.	108

Common Name(s)	Immobilization Method	Purpose of Study	Results and Comments	Ref(s)
Streptococcus olivochromogenes	Spores physically adsorbed on carriers. Carriers had pore sizes ranging from 0-195 μ .	To examine effect of pore size on cell accumulation	Bioassay by protein content of support. More negative carrier allows more growth but adsorbs fewer spores. Spore size 1-2.5 μ . Highest growth is pores $\sim$ 40 μ . Useful study on pore size requirements for different microbes, showing effect of reproduction method. Gives general guidelines which should apply to any type of cell.	102

Common Name(s)	Immobilization Method	Purpose of Study	Results and Comments	Ref(s)
Streptomyces	Polyacrylamide gel entrapment	To examine mass transfer kinetics of reactor/cell system for use in isomerization of glucose	Gel cast in thin slabs into vertical plate reactor. Feed 2.2M Glucose, 0.05M $MgSO_4$, 0.024M $CoCl_2$, 0.1M PO_4 buffer (pH 7) Temperature 60-75°C. Flow 30-90 cc/hr. Experimental data fitted fairly well by model including diffusion in homogeneous gel and dispersive flow in liquid w/no boundary layer resistance. M-M constants increase with temperature. Theoretical values are greater than experimental data because of boundary layer resistances. Good theoretical work but reactor not particularly useful for practical problems.	109

Common Name(s)	Immobilization Method	Purpose of Study	Results and Comments	Ref(s)
Streptomyces venezuelae	(1) casting and drying of collagen/cell mixture w/o or w/o cross link w/glut. (2) electroco-deposition of cell and collagen (3) impregnation of cells onto collagen w/ or w/o cross-link w/glut.	To examine collagen as a cell carrier for use in glucose isomerization	Polarimetry used to assess enzyme activity. Cells heat treated to inactivate undesirable proteins and to fix glucose isomerase inside cell. With residence time of 2.5 hrs, plug flow converts 40% of LM dextrose @ 70°, pH 7 for 15 days. Batch reactor converts 40% of LM dextrose in 6 hrs. over-long times, problems developed with membrane which were solved by casting membrane onto Vexar, a DuPont reverse membrane.	19

Common Name(s)	Immobilization Method	Purpose of Study	Results and Comments	Ref(s)
Cell #Sp-92 - Novo Enzyme Corp.	Entrapment in cellulose acetate fibers and membranes	To test use of immob. whole cells for glucose isomerase reactions.	Cellulose diacetate and triacetate used - dissolved in CH_2Cl_2 + H_2O and extruded into toluene or cast onto acetone on glass. Fibers ca. 200 - 500 μ . Membranes 10 - 25 μ thick Fibers show very low activity (2-7%) - diffusion limited. Membrane had up to 58% of free-state activity. Leakage of cells seen from both forms. Definite problems - there are better membrane formulations already available. Other fibers may be superior.	110

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