IMMOBILIZED BIOSYSTEMS

THEORY AND PRACTICAL APPLICATIONS

Edited by
I. A. Veliky and
R. J. C. McLean

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Theory and Practical Applications

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Theory and Practical Applications

PREFACE

This condensed view on immobilization of viable and non-viable cells, proteins, enzymes, active molecules and their interaction with the natural or synthetic carriers for performing biochemical and chemical reactions suggested a few thoughts for the present and future use of immobilized biosystems. It is essential that we understand the coordination of principles and theories of various scientific fields. Without understanding and using the basic functions in their broad integration many valuable applications and results of scientific research could be lost.

Even for Nature, it is difficult to combine all the necessary broad and detailed knowledge, imagination and creativity into one person's mind. However, there is a possibility of interlocking several minds and connecting the missing links between scientific fields and proceeding faster by transforming data and results into practice. Yet, one of the interlocking minds, the central coordinating mind, must have a powerful imagination and excellent background knowledge to be creative.

The above are generally applicable thoughts but are closely related and reflected in the topic of this book. Immobilized biosystems, as described by the authors, indicate examples of how the principles of integration work.

The first chapter describes a series of natural and synthetic carriers used for immobilization of viable cells and active organelles and molecules. The authors have shown the interaction and requirements of physico-chemical knowledge needed to interpret such a semi-synthetic biosystem.

The second chapter reaches even deeper into theory and indicates the potential of immobilized proteins for use as sensitive biosensors. The third chapter evolves the integration of physico-chemical principles into bioactive catalytic systems reflected by biochemical reactions. How to apply the preserved bioactivity of the immobilized viable cells and active proteins is demonstrated in Chapter 4. The authors suggested the use of glass fibers as support for the bioactive systems as inert, non-toxic and stable recoverable carriers.

The last chapter is a demonstration of another immobilized system in Nature. The authors describe a natural polymer produced by micro-organisms partially as a protection against environmental conditions. The polymer forming a biofilm inside the human body may carry and protect cells of micro-organisms difficult to fight with antimicrobial agents. In this case we have a natural immobilized system which we would like to 'dis-immobilize' in order to remove and cure the infection caused by the immobilized micro-organisms.

In this book, the described knowledge of immobilization techniques and characteristics of various natural and synthetic carriers for biochemical reactions is only a small indication of its great potential. The high concentrations of biocatalytic active molecules on the vast surfaces of many carriers offer their elevated interaction with the transforming substrate increasing the reaction and the yields. Among other benefits of the immobilized biosystems is the increased protection against physico-chemical environmental effects. Such batteries of biocatalytic units can perform in a number of cases with much higher capacities than the classical chemical technologies.

The present knowledge and applications of many immobilized biosystems are in their initial stages. Additional knowledge and understanding of many interactions between the active molecules and carriers are being gained. In the near future we shall see many new processes, medical treatments, transplants of active tissues and cells immobilized on inert carriers, detoxification, environmental-contamination detection and purification, all based on integrated knowledge of immobilization principles and their imaginative applications.

Ivan A. Veliky

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INTRODUCTION

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'Creativity is a clone of knowledge and imagination.'

It is mankind's natural behaviour to challenge the unknown. Most of us are constantly fascinated by unexplored phenomena and complexity in Nature, our daily lives and the environment. Progress and scientific achievements increase our curiosity and imagination. The human brain has extremely high potential and energy. It also has a built-in, self-activating and stimulating, exceptionally complex mechanism as the main source of accumulated knowledge and inherited imagination and creativity.

Accumulated knowledge as a result of learning may not always be sufficient to produce progress. To use the knowledge one must have imagination. This combination will result in creativity and eventually will bring new discoveries and stimulate progress in science and technology. Imagination helps to combine the bits and pieces of gathered knowledge into a logical, useful product.

Such combination of knowledge and imagination is essential for all scientific fields and research areas. One of the most complex and, on knowledge and imagination, demanding fields is biology. Living systems and their interaction with the environment have created a well balanced biosphere harmony. There is a tendency to relate this harmony to interaction within the living systems only. However, there is a structure, a foundation needed for initiating, preserving and supporting the functions of living systems. Such a structure has an essential importance for the existence and functioning of living systems. Therefore, a better comprehension of the principles of coexistence in our environment can have a significant effect on creativity in science and can motivate new achievements much faster. Where to look for such a motivation? Nature is a source of infinite information. Nature is the World's largest data bank of facts.

There are many examples around us to indicate the importance and role of supporting structures or carriers of living systems. All biosystems are actually attached to one or another kind of a carrier or structure made of inorganic or organic materials. Relative to the carrier or structure, they are recognized as the natural immobilized biosystems. There are macro- and micro-systems. There are immobilized tissues, cells and active molecules. All of those individual cells survive and perform better in the immobilized state. Trees grow with roots attached to soil, algae grow attached to solid supports such as stones in streams and lakes. Some cells or tissues grow on supporting carriers made of inorganic or organic polymers. The catalytic systems of cells – enzymes – are part of a protein or other polymeric structure. One can continue selecting examples from our environment and biosystems to indicate the importance of a carrier for life performance. On the pages of this book the reader will find a description of old, natural and new synthetic carriers, most of them simulating Nature's structures or physico-chemical laws. The chapters of this book describe the theory, application and use of the carriers for immobilization of bioactive molecules, cells or tissue.

The forms of association between Nature and immobilization of bioactive systems are numerous. This book offers selected examples for the immobilization or cells and enzymes and indicate some of the infinite applications and potential at present and in the future.

Without a doubt, the biosystems represent the only example of the most complex of coordinated chemical reactions which also have a built-in memory bank of every one of them. The reactions can repeat not only at the same rate, but also with the same precision by reproducing the same biosystem as selected from the DNA memory bank of data. We also know that biosystems by some standards and expectations may not be perfect. For example, yeast cells convert sugar into ethanol and carbon dioxide. During the process the cells divide, using some of the sugar molecules as energy source and building material for the new cells and, obviously, they produce by-products from the nutrients necessary for their survival and reproduction. Some of the by-products in the wine industry are important for the taste and bouquet which determine the wine's quality. In industry which produces ethanol in large quantities as a pure product, the lower yields and by-products are simply economical losses. To improve this process and increase the production rates, it was attempted to immobilize viable yeast cells in various polymers. The cells immobilized by encapsulation or entrapment in those polymers formed high concentrations of active units in relatively small areas. To prevent the cells from dividing and multiplying, they were fed with the limited

nutrient essential only for ethanol production. This nutrient control helped to increase the efficiency of producing ethanol of such immobilized biosystems when compared with the regular fermenters.

The viable cell immobilization approach is not always the best. Some polymers used for immobilization could allow leakage of cells and consequent clogging of bioreactors. Other polymers could slow down or prevent the transport of substrates or metabolites because of their molecular sizes. Such effects could lower the efficiency of a bioreactors. It is therefore important to select a suitable carrier.

In some simple cases a viable cell can be replaced by an immobilized non-viable cell form as an economical source of specific oxidases, hydrolyses, and other enzymes. A simple example would be the enzymatic hydrolysis of sucrose by invertase. The resulting products of this reaction are glucose and fructose. Many strains of Saccharomyces are known to produce enzyme invertase. To obtain non-viable cells as a source of invertase, *Saccharomyces cerevisiae* cells were homogenized to remove the viable cells. The homogenized mixture was immobilized by encapsulation in calcium alginate. A cylindrical bioreactor was filled with spherical beads containing encapsulated homogenate of non-viable cells with invertase activity. A continuous input of sucrose to the bioreactor resulted in continuous output of a mixture of fructose and glucose. The column was active for several weeks without replacing the immobilized homogenate. The advantage of such immobilized homogenate of cells (non-viable cells) or tissue is mainly in better stability of the enzyme in encapsulation. The enzyme is actually 'immobilized' on the non-viable structure of the cell or tissue and this complex is then encapsulated in natural or synthetic biologically inert polymer. Such 'double' immobilized systems could prevent leakage of enzymes for which the molecules are smaller than the pores of the gel used for encapsulation.

The example was completed by a demonstration of one potential practical application. A second bioreactor containing immobilized viable cells of another yeast strain known to utilize only glucose was fed the effluent of the first bioreactor containing glucose and fructose products from sucrose conversion by invertase. The glucose was utilized by the immobilized 'glucose-eating' yeast and converted to ethanol leaving fructose syrup as a by-product.

The demonstrated principle of immobilized biosystems based on enzymatic activities of non-viable and viable yeast cells could be used in practical applications such as fructose syrup production, fructose sweetened alcoholic beverages, dessert wines, etc. The process can be improved by replacing the immobilized non-viable yeast cells as the source of invertase

by purified and obviously much more highly active pure enzyme immobilized on another carrier. A number of options and techniques are described in this book.

The authors of the chapters in this book directly or indirectly indicate the ways and areas of potential benefits of the immobilization of bioactive molecules and complexes for industry, technology and medicine.

One naturally occurring immobilized biosystem in the human body is worth mentioning specifically. Some of the problems of treating human and animal chronic infections may result from encapsulation of infectious micro-organisms in polymers formed and excreted by the micro-organism on the body's tissue, organs or walls of body fluid transporting passageways. Details of such cases are described in one of the chapters in this book. Polymers produced by the micro-organisms form a film on tissue and create a 'hiding' place for the micro-organisms. The antimicrobial molecules are effective and kill only the freely floating micro-organisms in the body fluids. However, they cannot penetrate and reach the micro-organisms embedded or encapsulated in the polymeric substance. The molecular sizes or physico-chemical reactions between the antimicrobial compound and the polymer produced by the micro-organisms can alter their transport mechanism. Therefore 'hidden' microorganisms in the polymer will survive and may become a source of chronic infection. This example demonstrates one of the principles of how naturally occurring immobilized biosystems could protect micro-organisms against environmental effects.

It was indicated in general terms what are immobilized biosystems, how they function and participate in biocatalytic reactions and simulation of complex biochemical processes performed in living systems. There are numerous books on the market at present and more will be coming out with details on immobilization of cells, enzymes and their combination for various applications. Although Nature is the best teacher and the source of infinite information, it is not necessarily perfect. It is Man's natural desire to learn, and continually improve his or her knowledge. The biosynthetic processes in Nature can produce healing products for many if not all diseases. Most of the biosynthetic processes are performed in plants as well as animals and microbes. Such biosynthetic processes are lengthy, multistep procedures. However, they are not necessarily the most efficient process. The biosynthesis of an active compound can sometimes be improved when performed outside the living system. It may be possible to dispose of a side reaction not essential for obtaining the final product, whereas in the living system the side reaction may be necessary as an intermediate protective step. Therefore, in a care-

fully designed artificial system, the biosynthetic process can run more efficiently and produce elevated yields and purity of the final product.

A good example of improved biosynthetic activity is the oxidases of certain cells. Plant cells isolated from carrot root perform bioconversion of cardiac glycosides (digitoxigenin, gitoxigenin) by introducing a hydroxyl group into the C_5 position. This reaction is difficult to perform by a classical chemical process. The biosynthetic process using viable and non-viable immobilized cells is efficient and provides a yield of over 80 per cent. This yield cannot be reached by present synthetic chemical methods. Also, the hydroxylated products of genins are most stable in the human body. Their therapeutic dose for heart patients is easier to determine than in the case of the non-modified digitalis drugs.

There are certainly many other reasons why and where the immobilized system could apply in practice. The immobilized cells or tissue have an important place in medical practice. The immobilized cells or tissue can replace cells, tissue or even glands damaged by illness or the aging process, or can replace non-developed or damaged brain cells, etc. Why immobilized cells or tissue? Why not a simple transplant of healthy tissue only? In some cases a simple transplant of tissue may be sufficient. The advantage of using immobilized cells for such repairs of damaged body organs is in preventing rejection and increasing stability and location of the transplanted cells or tissue. Before a transplant of the healthy cells is done, the cells are attached to an inert carrier and cultured in the medium gradually enriched with the body fluids of the recipient. The donor cells grow attached to the carrier and gradually adapt to the conditions of the recipient's body, hence reducing the rejection to a minimum. This cell conglomerate, an artificially created 'mini-organ' or 'mini-gland', is then used to replace the damaged human tissue.

Production of active medicines and drugs could be improved by using more efficient systems of immobilized active cells (microbial, plant or animal) in batch or continuous bioreactors. Fermentation processes could be controlled by using immobilized enzymes as biosensors for detecting metabolite concentration and the supply of substrates and nutrients. This book has a few innovative demonstrations in the construction and use of biosensors.

Immobilized viable cells in fermentation processes in the food industry are well recognized. The ancient way of vinegar production from wine or diluted alcohol is based on simple immobilization of bacteria on wood chips. There are other places in the food and fermentation industry, such as the production of fructose syrup, ethanol and other solvents, where immobilized enzymes or viable cells are used. The cheese-making industry is

also using immobilized enzymes for processing milk into various cheese products. The process is continuous and preserves very well the flavour of the final product.

An open area for application of immobilized systems is the agriculture industry. Biological control of pests in agriculture is on the rise. Improvement of the propagation of predators has yielded good results by using nutrient saturated carriers.

The few abbreviated notes on the usefulness of immobilized biosystems in research and development indicates the need to challenge the unknown in this field. The applications of the findings and discoveries in the broad spectrum of the complexity of immobilized biosystems are yet to be recognized.

ERRATUM

The following section is missing from Chapter 1, p. 19.

Because the alignment of individual cellulose chains is frequently imperfect, irregularities in fibril structure occur. This is reflected in the structure of the whole fiber which consequently consists of polycrystalline aggregates separated by amorphous regions (Hon, 1988).

Cellulose was one of the first materials to be used as a matrix for covalent binding of enzymes. A survey of methods reported in the past 10 years (Kennedy, 1978; Sturgeon, 1982; White, 1985; Kennedy & Cabral, 1987*a*; Gemeiner *et al.*, 1989*a*) showed, however, that cellulosic materials are not as popular as other polysaccharide supports. The reasons are susceptibility to microbial degradation and nonspecific adsorption properties. Nevertheless, cellulose has a major advantage over other polysaccharide supports in that it has become available in many different physical forms, such as fibers, microgranules, micro-crystals, beads, gel particles, membranes, tubings, emulsions, etc. For industrial reactors, less pure cellulosic material can be used in the form of ropes, pulps, chippings, cloths or papers.

A variety of morphological properties of cellulose should be assessed with respect to its specific application prior to selection for evaluation. Sufficiently adequate for full characterization of carrier properties of cellulose are parameters such as surface area and pore diameter. Both of these parameters affect in turn the loading of cellulose by the enzyme. To take account of these parameters celluloses can be very roughly classified as low-porous and high-porous matrices based on their morphology.

1.3.1.1 Low-porous Celluloses

Low-porous celluloses available on the market are designed mainly as fibrous, powder, microgranular and microcrystalline. Cellulose powder is prepared either by mechanical grinding of fibrous cellulose or by its heterogeneous hydrolysis with mineral acids.

CONTENTS

Chapter 1

NATURAL AND SYNTHETIC CARRIERS SUITABLE FOR IMMOBILIZATION OF VIABLE CELLS, ACTIVE ORGANELLES, AND MOLECULES

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