PROTEIN IMMOBILIZATION

Fundamentals and Applications

edited by
Richard F. Taylor

Protein Immobilization

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Edited by
Richard F. Taylor
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Series Introduction

Bioprocess technology encompasses all of the basic and applied sciences as well as the engineering required to fully exploit living systems and bring their products to the marketplace. The technology that develops is eventually expressed in various methodologies and types of equipment and instruments built up along a bioprocess stream. Typically in commercial production, the stream begins at the bioreactor, which can be a classical fermentor, a cell culture perfusion system, or an enzyme bioreactor. Then comes separation of the product from the living systems and/or their components followed by an appropriate number of purification steps. The stream ends with bioproduct finishing, formulation, and packaging. A given bioprocess stream may have some tributaries or outlets and may be overlaid with a variety of monitoring devices and control systems. As with any stream, it will both shape and be shaped with time. Documenting the evolutionary shaping of bioprocess technology is the purpose of this series.

Now that several products from recombinant DNA and cell fusion techniques are on the market, the new era of bioprocess technology is well established and validated. Books of this series represent developments in various segments of bioprocessing that have paralleled progress in the life sciences. For obvious proprietary reasons, some developments in industry, although validated, may be published only later, if at all. Therefore, our continuing series will follow the growth of this field as it is available from both academia and industry.

W. Courtney McGregor

Preface

This book presents a practical overview of the technologies used to immobilize proteins, together with examples of specific applications for immobilized proteins.

Rather than being limited to immobilized *enzymes,* this book focuses on immobilized *proteins.* This emphasis is aimed at drawing attention to the increasing importance of immobilized antibodies, structural proteins, and macromolecular complexes (such as cellular receptors) to the development of new immobilized, protein-based products.

Immobilized enzymes (and cells containing desired enzymes), however, continue to command the most attention in the development and commercialization of immobilized proteins. From the first adsorption immobilization of invertase onto charcoal and alumina by Nelson and Griffin in 1916, through the intense research activities on enzyme immobilization in the 1960s, immobilized enzymes emerged in the 1970s as a major, commercially viable technology for food and drug processing. Today, commercial processes routinely utilize immobilized enzymes such as glucose isomerase, glucoamylase, penicillin amidase and acylase, invertase, lactase, fumarase, amino acylase, aspartase, and hydantoinase. In one case—immobilized glucose isomerase—the production of high-fructose syrups from dextrose syrups almost exclusively utilizes the immobilized enzyme.

From the base technology derived from enzyme immobilization, other

immobilized proteins are being developed and applied in commerce. Immobilized antibodies form the basis of modern clinical diagnostics and are being applied to new detection and diagnostic devices, such as biosensors, for applications in medicine, food and drug processing, and environmental monitoring. Immobilized peptides and proteins that bind toxic substances are being developed for detoxification applications. New therapeutics are being developed that utilize antibody–toxin conjugates to target and destroy tumor cells. Immobilized cellular receptors, such as receptors isolated from nerve tissue, are being used as the basis for a new generation of diagnostic biosensors. Immobilized antibodies and binding proteins also serve as the basis for biospecific separations, such as affinity chromatography and filtration, which are being applied to the production of new genetically engineered products such as drugs and hormones.

These examples illustrate the increasing uses for immobilized proteins. Key to such use is our better understanding of protein immobilization technology and the application of this technology to useful products and processes. In a world environment where basic research and development is more than ever driven by final, commercializable products, protein immobilization still provides the researcher and inventor with research challenges as well as product development rewards.

This book is aimed at readers who carry out basic research and development in protein immobilization, as well as those who utilize protein immobilization technologies in their processes and products. No text can be inclusive of an area as diverse as protein immobilization. Our aim was to present discussions of both basic and applied aspects of protein immobilization. If, at the very least, the information presented in this text stimulates the reader to further studies in development of protein immobilization methods or in the application of immobilized proteins to new processes or products, it will have achieved its goal.

Richard F. Taylor

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Introduction: The Current Status of Immobilized Protein Technology

Richard F. Taylor

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I. INTRODUCTION

The last half of the twentieth century has witnessed a revolution in the practical application of the biological and physical sciences. The merging of disciplines grounded in chemistry and biology with others in physics and electronics has spawned new technologies and products. Of such interdisciplinary technologies, the new biotechnology (as distinct from "old" biotechnology grounded in fermentation processes stretching back to ancient times) stands out as a primary example. To practice the new biotechnology, expertise may be required in disciplines previously thought revolutionary in their own right, such as biochemistry, molecular biology, genetics, biophysics, and microelectronics. The primary difference which has brought biotechnology into our daily lives is that its practice is leading to practical products.

Just as the practical application of physics in the first half of this century resulted in controllable nuclear power and the electronics revolution, biotechnology is being used today to change the fabric and quality of our daily lives. Genetically engineered drugs such as insulin, human growth hormone, α-interferon, and tissue plasminogen activator (TPA) are available and promise to increase the quality of health care. New vaccines such as those for hepatitis B, scours, rabies, and coccidiosis are being produced in recombinant microorganisms or by solid-state peptide synthesis. Trans-

genic plants and animals are being developed to impart herbicide resistance and improve meat production, respectively. Improved methods are being developed for the production of specialty chemicals for the food and processing industries including recombinant enzymes (e.g., α-amylase, rennin, and lipase) and synthetic sweeteners (e.g., aspartame).

The art and science of immoblizing proteins is a biotechnology, and the oldest of the new biotechnologies. The use of immobilized enzymes for production of products such as sugars, amino acids, and drugs predates by nearly two decades biotechnologies such as genetic engineering and monoclonal antibodies. In addition to use in processing, immobilization techniques represent a base technology which is necessary for many biotechnology products. These include immobilized antibodies and enzymes used in diagnostic products; immobilized antibodies and binding proteins used in separation products such as those for bioaffinity chromatography; and immobilized proteins (enzymes, antibodies), protein complexes (receptors), and cells used for biosensors. Immobilization technology, then, is a key technology for the successful application of biotechnology to new products.

II. APPLICATIONS AND MARKETS FOR IMMOBILIZED PROTEINS

A. Applications

The primary application for immobilized proteins is the use of immobilized enzymes as catalysts in industrial processes and products. The high specificity, high rates of reaction, nontoxicity, water solubility, biodegradability, and use under mild conditions of pH, temperature, and pressure are major advantages over inorganic catalysts. For this reason, the use of immobilized enzymes is firmly established as an effective and economically favorable approach for the production of products such as fructose, synthetic penicillins, and amino acids. Specific examples of these processes are presented throughout this text.

While many texts have been written on immobilized enzymes, the focus of this text includes not only immobilized enzymes but other proteins which function through means other than catalytic activity, e.g., antibodies, receptors, and binding proteins such as proteins A and G, lectins, and avidin. These noncatalytic proteins are the focus of the most rapidly growing area of immobilized protein research, development and application.

As shown in Table 1, modern biotechnology needs have stimulated new applications of immobilized proteins. Of these, the areas being most affected by improvements in immobilized protein technology are bioseparations, diagnostics, bioprocessing, and new disease therapies.

Table 1 Immobilized Protein Application Examples

Application	Immobilized Protein(s)	Support	Ref.
Research Applications			
Artificial photosystem	*Desulfovibrio vulgaris* hydrogenase	Nylon gel	18
Photoreaction systems	Spinach photosystem II submembrane fraction	Cross-linked albumin membrane	19
Drug metabolism studies	Various metabolism enzymes	Agarose, sepharose, acrylamide, etc.	20
Sialic acid assay	*N*-Acetylneuraminic acid aldolase and lactic dehydrogenase	Gelatin membrane	21
Diagnostic Applications			
Analytical bioassays	Bacterial luciferase	Nylon, glass and agarose beads, etc.	22
DNA analysis	Restriction endonucleases	Nylon, cellulose, gelatin, and agarose	23
Hydrolysis of nerve gases	Acetylcholinesterase	Agarose beads	24
Detection of cholinergics	Acetylcholine receptor	Polymeric membrane	25
Medical Applications			
Fibrinolytic polymers and vascular prosthesis devices	Urokinase, trypsin, and streptokinase	Dacron-reinforced collagenous tubes, polyvinylidene fluoride films, nylon, etc.	26–28
Removal of drugs, toxins, antibodies, etc., from blood	Appropriate antibodies and binding proteins	Agarose beads, etc.	29
Intracorporeal treatment of gout	Uricase	Polyethylene glycol	29
Extracorporeal removal of asparagine in cancer patients	Aspariginase	Methacrylate plates	30
Inhibition of platelet adhesion to medical prosthesis devices	Albumin–heparin complex	Polyvinyl chloride and other polymers	31
Extracorporeal heparin removal	Heparinase	Agarose beads	32
Plasmapheresis immunotherapy	Antibodies to IgE	Porous cellulose, agarose, and glass beads	33

Table 1 (Continued)

Application	Immobilized Protein(s)	Support	Ref.
Industrial Applications			
Sterilization of dairy products	Catalase	Collagen spiral membrane reactor	34
Tallow hydrolysis	Lipase	Microporous acrylic membrane reactor	35
Aglycone production from glycosides	Naringinase	Controlled pore glass	36
Nucleotide production	T4 Polynucleotide kinase	Sepharose beads	37
Oxygen extraction from seawater	Hemoglobin	Polyurethane	38
Purification of immunoglobulins	Proteins A and G	Vinyl-cellulose spiral membrane cartridge	39
Sugar production from hemicelluloses	Cellulases	Controlled-pore glass, alumina, and titania	40
Juice clarification	Endo-polygalacturonase	Trimethylchitosan and others	41
Milk lactose hydrolysis	β-Galactosidase	Polyvinyl chloride-silica spiral flow reactor	42

Immobilized antibodies and binding proteins (such as concanavalin A and protein A) are widely used for separation and purification of materials by affinity chromatography and filtration (1,2). Improvements in the stability of support matrix materials and coupling methods have led to more rapid affinity chromatography methods, i.e., high performance affinity chromatography (3–5) and new membranes for fast flow affinity filtration (6). The use of immobilized protein affinity supports in two-phase aqueous systems is also being developed for application to process scale separations (7). While this method has, to date, primarily utilized affinity dyes bound to polyethylene glycol for purification of enzymes (such as dehydrogenases and kinases)(8,9) from salt or dextran second phases, other workers are developing two-phase systems for immunopartitioning using immobilized antibodies to purify enzymes, cells, and hormones (10,11). Examples of support materials used for affinity separations and their application to enzyme immobilization will be discussed in Chapter 4 of this book.