Biological Systems Engineering

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Biological Systems Engineering

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Foreword

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As a rule, only original research papers and original review papers are included in the volumes. Verbatim reproductions of previously published papers are not accepted.

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Preface

What many think of as modern biochemical production technology began in the early 1940s, as an effort to meet the increasing demand for penicillin during World War II. During that time, penicillin production moved from surface culture in milk bottles to submerged culture in tanks as large as 100,000 L. In the 60 years since then, significant advances in biochemical production technologies have occurred, as dramatic advances have been made in our understanding of cellular systems, and how these systems can be manipulated to produce desirable products. Today, production of biochemicals includes not only traditional subjects such as fermentation engineering and applied microbiology, but subjects as diverse as recombinant DNA technology, metabolic engineering, genomics, and combinatorial chemistry (just to name a few). This field continues to evolve as new technologies become available to operate, monitor and control bioprocesses, and as new biological discoveries are made at an ever increasing pace.

The majority of the papers contained in this text were presented at the PacifiChem 2000 conference, held in Honolulu, Hawaii in December 2000. Our goal in organizing this symposium and in compiling this book was to bring together scientists from around the Pacific Rim who are using various modern biological tools to study various aspects of biochemical production. The book is divided into three sections: (1) microbial and enzymatic systems, (2) animal cell systems, and (3) combinatorial engineering and analytical methods. Expression systems discussed in various chapters are used for the production of organic acids, specialty sugars, enzymes, and antibodies. Our hope is that this book will benefit the reader by providing a glimpse of how modern biological tools are being applied to the production of a mixture of biochemical products.

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Biological Systems Engineering: An Overview

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Biological systems have been used for thousands of years to produce a wide variety of products. These historical processes primarily involved microbial fermentation, and were used to produce food and alcohol. In the last 50 years, scientific advances have allowed the use of a wide rage of biological systems, that include not only microbial fermentation, but culture of animal and insect cells, use of enzymatic systems, and combinatorial biochemistry. These approaches have been employed to produce a broad range of products that span the spectrum from high value pharmaceuticals to commodity chemicals. This chapter gives an overview of some of biological systems currently used, the engineering of these systems, and the products they are used to produce.

Microbial and Enzymatic Systems

With the introduction of recombinant DNA technology, a completely new approach to the development of biochemical processes became possible. Various genetically modified microorganisms are currently used for the production of not only recombinant proteins but also non-protein compounds. The recombinant protein products include vaccines, therapeutic agents, and animal growth hormone. Recombinant DNA techniques have also enhanced the production of a broad range of low-molecular-weight non-protein compounds including amino

acids, antibiotics, vitamins, and dyes. Genetically modified microorganisms are also used to produce biopolymers that are useful in the food-processing, manufacturing, and pharmaceutical industries. Various kinds of microorganisms are currently being used as biological factories for the production of such commercial products. Among the microorganisms, *Escherichia coli* and *Saccharomyces cerevisiae* have been widely used as host microorganisms, since *E. coli* is the most studied organism and *S. cerevisiae* is a well-studied nonpathogenic eukaryotic microorganism.

For the production of commercial products, microbes should be cultivated in large quantities under conditions that give maximal productivity. This requires the development of high-expression host cell/vector systems, which can be regulated and are stable in large-scale processes. High cell density culture is one way to increase productivity. In high cell density culture, one major problem is the formation of undesirable by-products. For example, in *E. coli* culture acetic acid is a major inhibitory by-product to cell growth, and is produced when cells are grown under oxygen-limiting conditions and in the presence of excess glucose. The best way to obtain high cell density is to use a fed-batch fermentation strategy, and as a result fed-batch operation is one of the most popular modes of operation for the industrial production of recombinant proteins. Various feeding strategies for fed-batch operations have been proposed to obtain a high cell density and high concentration of recombinant proteins. These include feeding strategies using pH-stat, DO-stat, glucose-stat, and controlling the specific growth rate.

Gene manipulations either create a new pathway or augment a preexisting pathway for the production of a specific compound. The production ability of microorganisms could be improved more systematically by metabolic engineering (*1*). In contrast to the prior focus on single reactions in a pathway, metabolic engineering concerns the enumeration and quantification of intracellular metabolic fluxes, and emphasizes the integrated metabolic networks. This systematic and integrated approach can increase product yield and productivity, and can also provide the formation of novel biochemical products. More global cellular regulatory data are accumulating with the current development of functional genomic and proteomic technologies. This will provide more detailed information for the biochemical production.

Enzymatic systems have also been widely used in a large variety of industrial applications such as production of high-fructose syrups from corn starch, enzymatic hydrolysis of lignocelluloses to monomeric sugars, manufacturing of L-amino acids by resolution of racemic amino acid mixtures, manufacture of semi-synthetic penicillins and even production of commodity

chemicals like acryl amide. Important aspects of enzymatic system development are to obtain suitable enzyme with high activity and stability, to improve the properties of enzyme, and to design optimal bioreactor system for enzymatic reaction and product separation. As for screening of enzymes, microorganisms in extreme environments such as high temperature, salt concentration, pressure and alkaline pH, have recently drawn much attention as good sources, as enzymes from these microorganisms possess higher stability and broader substrate specificity compared to those from microorganisms from moderate environments.

Artificial evolution techniques, or so called "directed evolution" has been utilized to improve the properties of a number of enzymes *(2)*. This method has emerged as a powerful alternative to rational approaches for enzyme engineering. Directed enzyme evolution typically begins with the creation of a library of mutated enzyme genes, which are created using mutagenesis methods such as error-prone PCR, oligonucleotide-cassette mutagenesis and DNA-shuffling. Selection or rapid screening is then used to identify improved enzymes with respect to the desired property. The genes encoding these enzymes are subsequently subjected to further cycles of random mutagenesis, recombination and screening in order to accumulate beneficial mutations. As a result of enzyme screening from extremophiles, or directed enzyme evolution, enzymes with high activity, thermostability, substrate specificity, enantioselectivity and organic solvent tolerance have been obtained. These enzymes will contribute to the development of green chemical process.

Animal Cell Systems

Although microbial systems offer many advantages for the production of biochemical products, they have limitations in the production of glycoproteins. Many animal cell systems are able to glycosylate proteins in a fashion that is reasonably similar to the way in glycosylation occurs in a human being. Biological properties of the glycoproteins may depend on the glycosylation pattern, which also affects the therapeutic activity of pharmaceutical glycoproteins. Therefore, many pharmaceutical glycoproteins are produced in recombinant animal cell culture. Mammalian cell and insect cell systems have been widely used for the production of glycoproteins.

The insect cell-baculovirus system has received rapid and wide acceptance as an alternative to classical bacterial or yeast systems for the production of recombinant proteins. This system has several advantages, including high expression owing to a strong polyhedrin promoter, production of functionally

and immunogenetically active recombinant proteins due to proper post-translational modifications, and nonpathogenicity of the baculoviruses to vertebrates and plants (*3*).

The key issues of the animal cell system have been concerned with the development of high-expression host cell/vector systems, the high cell density culture technology on a large-scale, the development of an efficient process for the production of recombinant proteins or viruses, and media development including low serum and serum-free media.

For the efficient production of cloned-gene protein, the high rate of cloned-gene expression and the viability of host cell are important factors. The increased host cell longevity favors the longer production of recombinant proteins, which results in higher productivity. Cell death can occur by either necrosis or apoptosis. This depends on the level of stress experienced by the cells. In animal cell culture, the decrease in cell viability is a consequence of apoptosis, which is a feature of many commercially important animal cell lines. Apoptosis is triggered by infection with viral vectors, suboptimal cultivation conditions, or heterologous gene expression, resulting in the lower process productivity (*4*). To suppress apoptosis in cell culture processes, three basic approaches have been conducted. Those are elimination of nutrient deprivation, addition of apoptosis-inhibiting compounds and over expression of apoptosis suppressor genes.

Combinatorial Bioengineering and Analytical Methods

Since the early 1990's, one of the key goals of combinatorial chemistry has been to revolutionize drug discovery. In the time that has elapsed, combinatorial methods for generating molecular libraries, coupled with high-throughput screening, have become core technologies for the identification of ligands to receptors and enzymes. The identified ligands can be utilized as powerful tools for pharmacological studies, and are essential as lead compounds for drug development.

Combinatorial libraries of small organic molecules can be generated by a variety of synthetic methods. For example, combinatorial libraries composed of completely random sequences of peptides or oligonucleotides can be synthesized by solid-phase synthesis with mixtures of activated amino acids or nucleotides in randomized coupling. Libraries consisting of random, site-directed mutants of a specific protein or nucleic acid oligonucleotide are composed of many variants of an initial parental molecule and are generated by biological machinery such as

cells, phage, PCR and cell free protein synthesis systems. The phage display, cell surface display and ribosome display systems can provide combinatorial libraries with a pair of DNA and corresponding peptide or protein.

In any combinatorial library composed of peptides or oligonucleotides, all structures are built from a common set of chemical building blocks, with each molecule possessing a unique combination or sequence of these building blocks at each synthetically incorporated position. Additionally, the molecules all possess a common structural core or synthetic linkage, dictated by the type of molecules in the library and by the actual synthetic strategy employed. For example, collections of peptides or protein molecules in a combinatorial library are usually built from the 20 naturally occurring amino acids, and possess a common synthetic linkage, an amide bond between each position in the polymeric molecule. Thus the synthesis of combinatorial libraries composed of peptides or oligonucleotides can be done in high-throughput and systematic fashion.

Combinatorial approaches have been most successful when a semi-rational approach has been used to design the library of molecules to be prepared and tested. In these efforts, libraries are designed by using knowledge of the mechanism or structure of the biological target, or by basing the library upon lead compounds that have previously been identified to bind to the biological target. Unfortunately, structural or mechanistic information for many biological targets is either unavailable, or does not provide sufficient insight to enable productive library design. Additionally, for many targets, lead compounds have not yet been identified or novel motifs for binding are desired. Thus, the route from design and synthesis of compound libraries to identification of biologically valid lead structure is still long and tedious. To overcome this situation, highly parallel approaches, including system automation, are required not only in synthesis but also in analysis and screening. Creating an efficient interface between combinatorial syntheses and bioassay is indispensable to satisfy these demands. For example, screening in the interior of resin beads, that of microarrays and label-free affinity binding detection have been developed *(5)*.

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