SEPARATIONS FOR BIOTECHNOLOGY 2

Edited by D.L.PYLE

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D. L. PYLE

Biotechnology Group, University of Reading UK

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Preface

The challenge of bioseparations is to isolate and purify identified products from the dilute product broth produced from cell culture. Innovation in bioseparations technology is increasingly driven by the requirements imposed by the growing importance of production on a process scale of injectable-grade products, and economic pressures to improve the efficiency of downstream processing. As in other areas of technical change, science does not necessarily precede new technology: progress results from a complex and messy mixture of advances in understanding, ingenious ideas, novel techniques and chance discoveries. What is certain is that close interaction between academics and practitioners, biological scientists and process engineers is needed to solve the problems of bioseparations. The Second International Conference on Separations for Biotechnology at Reading, UK, in September 1990 set out to provide a critical multidisciplinary forum for the discussion of bioseparations. This volume contains the papers presented at the meeting.

The meeting was organised around six themes with oral and poster presentations on the science and practice of bioseparations technology, and the same structure has been kept for this book. We have also included the texts of the keynote review paper by Professor Alan Michaels and the introductory review papers specially commissioned for the conference. Within each part of this book the review paper is followed by the contributed papers grouped alphabetically by their first author. All the original papers published here were accepted for publication after scientific refereeing.

This book would not have been possible without the help, unsung and invariably unpaid, of many colleagues. I am especially grateful to the SCI, the Organising Committee—the session chairmen in particular—and to the many anonymous reviewers. I owe a special debt to Tom Arnot and Gary Lye for their sterling work on the index and, finally, to Jean Davis for her outstanding and patient secretarial support in creating order out of paperwork whose complexity rivalled the biological soup which provides the basis for this volume.

D. L. PYLE

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FRONTIERS OF BIOSEPARATIONS TECHNOLOGY: UNSOLVED PROBLEMS AND NOVEL PROCESS CONCEPTS

Alan S. Michaels
President, Alan Sherman Michaels, Sc. D., Inc.
Chestnut Hill, Massachusetts
and
Distinguished University Professor, Emeritus
North Carolina State University
Raleigh, North Carolina

INTRODUCTION

The unique properties, and formidable processing problems associated with the isolation and purification of bioactive proteins and other biologically derived substances have become a major challenge to the chemical and biochemical engineer. The extraordinarily high standards of purity demanded of such products to satisfy regulatory requirements of safety, coupled with their dependence upon precisely controlled tertiary and quarternary structure for proper biological activity, require the development and use of separation/purification techniques uncommon to traditional bioprocessing practices, and of novel processing concepts which call upon the collective expertise of life scientist and engineer.

The basic separation strategies employed for bioproduct recovery are well-recognized, and generally observed in all bioprocessing operations today. These comprise (1) separation of the product(s) of interest in relatively impure form and low concentration from the biomass (either intact cells or cell debris) which produced them; (2) concentration of the product-bearing extract; (3) separation of the product in relatively high purity and still higher concentration from the majority of the adventitious impurities; and (4) isolation of the product from all but miniscule amounts of inactive or potentially toxic biologically-derived impurities. However, of the alternative technologies available for pursuing these strategies today, few are ideal for these purposes. Their limitations include poor product yield, inadequate separation selectivity, loss in product bioactivity due to denaturation or other structural change, inadequate process biosafety, or excessive processing cost. Efforts to circumvent these limitations have involved both attempts to

modify existing separations processes, and development of radically new processes better suited to meeting these stringent requirements. Some recent examples of these efforts as summarized below.

RECOVERY OF INSOLUBLES

In the area of solid/liquid separations for recovering extracellular bioproducts from whole cell suspensions, or intracellular products from dispersions of lysed cell fragments, UF/MF membrane separation processes continue to receive much attention. The recent development of surface-modified microfiltration/ultrafiltration membranes which resist fouling by biopolymers and colloids has been noteworthy, as has the development of novel fluid-management strategies (e.g., pulsatile flow, rotating-cylinder devices) which markedly improve the flux-performance, separation efficiency, and service lifetime of UF/MF separation systems. Of particular promise has been the development of ceramic and ceramic/metallic composite MF/UF membranes, which display unique fouling resistance, facile cleanability and sterilizability, and extraordinary durability.

Where genetically engineered proteins form inclusion bodies within recombinant organisms, and conventional solid/liquid separations are ineffective, it has been found that, by carefully controlled cell lysis, appropriate control of suspension pH and ionic strength, and precisely monitored centrifugation, efficient recovery of the inclusion bodies with relatively little contamination by cell fragments and other contaminants can be accomplished.

A novel process which involves treatment of a whole fermentation broth by fluidization with a selective particulate adsorbent permits efficient simultaneous recovery and purification of an extracellular metabolic product without need for any dewatering step. This process lends itself particularly well to cell-recycle, and semicontinuous whole broth extraction.

A unique single-step combination of membrane filtration and adsorption has been demonstrated: This makes use of a hollow fiber membrane ultrafiltration module, the shell space of which is filled with a particulate selective adsorbent; a cell-suspension containing a low concentration of desired protein is recirculated through the fiber lumens, with a small permeate stream being removed from the shell space. The cell-free, protein-bearing permeate is stripped of protein by the adsorbent; when the adsorbent is saturated, the shell-space of the module is flushed free of permeate, and eluted with saline or other displacing mixture to release and permit recovery of the purified protein.

EXTRACTION OF PROTEINS

Two-phase aqueous separation techniques for recovery of proteins from whole broths and lysed cell slurries continue to receive intensive study. A broadening selection of water soluble polymers and hydrocolloids, which yield improved selectivity and phase partitioning, and are economically practicable, should make this technology increasingly useful for large-scale protein recovery. The use of surfactants which micellize in non-aqueous media, and which in the micellar state are capable of sorbing specific proteins from aqueous solution, are the basis for the novel process of "inverse micelle" liquid/liquid separation for protein recovery and purification. When combined with the relatively new process of "membrane solvent extraction", which utilizes microporous membranes to greatly facilitate liquid/liquid extraction of "dirty" or emulsion-forming feedstreams, these techniques may constitute an important new dimension in bioproduct recovery.

ADSORPTIVE SEPARATIONS

A simple procedure for removal and isolation of a single protein species from a complex biopolymer mixture remains a major goal of modern separation technology. While preparative elution chromatography continues to be the preferred procedure from the standpoint of ultimate product purity, the complexity, tediousness, and high cost of the process are powerful deterrents to its use for industrial scale applications. Moreover, in many cases where column chromatography does permit efficient product recovery, the conditions of loading or elution often result in complete or partial denaturation of the protein of interest; this is tantamount to winning the battle and losing the war. The problems of restoring a denatured protein to its active, natural conformation (about which more below) are sufficiently apprehending to encourage the process engineer to seek other less drastic alternatives.

Recently, there has been rapidly increasing interest in the use of functionalized, selective solid-phase adsorbents for treatment of aqueous multicomponent protein solutions for the sorptive removal of one (or several members of a common class of) protein. In this process (sometimes termed "flash chromatography" or "bind/release"), the solution is contacted with the adsorbent until the solid is virtually saturated with the adsorbate, the solid phase flushed free of unadsorbed solutes, and then treated with a desorbing solution which displaces the adsorbed species in concentrated form, and regenerates the adsorbent. The solid phase may be functionalized in a number of ways: Attachment of ionogenic functions renders it an ion exchanger, which can differentiate between proteins of differing isoelectric points; attachment of "generic" affinity ligands such as triazine dyestuffs can render it selectively sorptive for certain classes of enzyme; attachment of cell