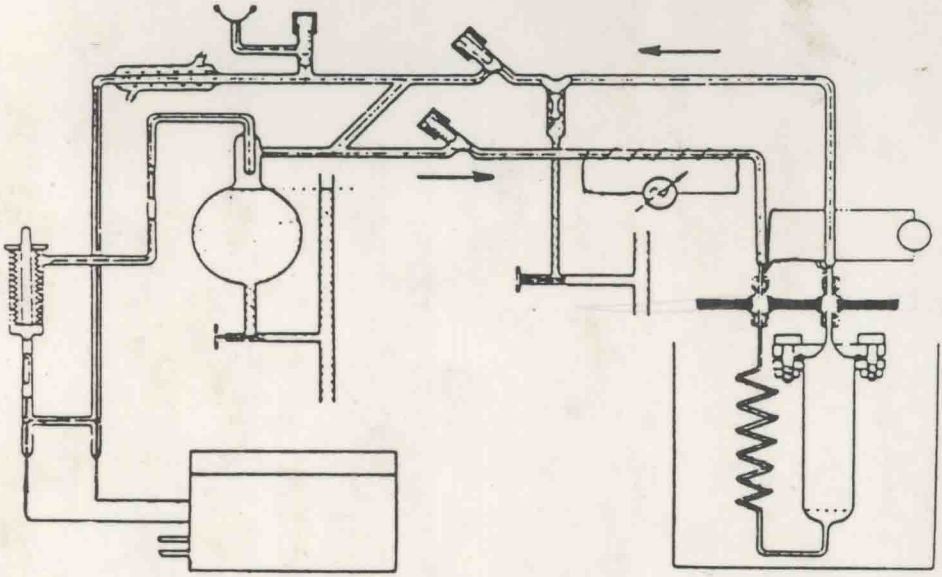
SEPARATION AND PURIFICATION TECHNIQUES IN BIOTECHNOLOGY



by
Frederick J. Dechow

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江苏工业学院图书馆
藏书章

np NOYES PUBLICATIONS
Park Ridge, New Jersey, U.S.A.

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Library of Congress Catalog Card Number: 88-34502
ISBN: 0-8155-1197-3
Printed in the United States

Published in the United States of America by
Noyes Publications
Mill Road, Park Ridge, New Jersey 07656

10 9 8 7 6 5 4 3 2 1

Library of Congress Cataloging-in-Publication Data

Dechow, Frederick J.
Separation and purification techniques in biotechnology.

Includes bibliographies and index.
1. Biomolecules--Separation. 2. Biomolecules--Purification. 3. Biotechnology--Technique. I. Title.
TP248.25.S47D43 1989 660.2'842 88-34502
ISBN 0-8155-1197-3

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Preface

Sorptive separation techniques are found in almost every product separation or purification scheme treating fermentation or biochemical feedstreams. These sorptive techniques include adsorption, ion exchange and liquid chromatography on solid supports. The major objective of this book is to place these different methods in perspective, relative to each other, so that selection of the appropriate technique or combination of techniques may be readily made. While the emphasis has been placed on laboratory evaluation techniques, the scale-up of these techniques and their industrial applications; it is hoped that sufficient theory has been provided so that the interested reader may use the references to pursue the selectivity and kinetic considerations for sorptive procedures.

The first chapter provides a brief sketch of the nature of the biochemical feedstream and all the processes which might be involved in isolating the desired products from that feedstream.

Chapter 2 covers adsorptive separation, which is the oldest of the sorptive techniques. While many people now regard adsorption as strictly for the removal of unwanted impurities or color bodies, this chapter shows that there are many applications where adsorption is useful in isolating biochemical products. The theory of column processes developed in this chapter is also applicable to the column operations for the sorptive processes described in the other chapters.

Ion exchange procedures are described in Chapter 3. This chapter builds upon what was presented in Chapter 2 by demonstrating the effects of the additional sorptive specificity associated with the exchange of ions. Operating parameters and equipment developed for water treatment and metal recovery applications have also been included since fermentation broths have characteristics which may benefit from the use of resin-in-pulp, fluidized bed and the other procedures presented.

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Chapter 4, Column Chromatography Processes, covers the use of sorptive materials to create an environment that allows the separate recovery of two or more solutes. The biospecific recognition of a solute for a ligand attached to the column material is covered in the last chapter on Affinity Chromatography.

It is my hope that this book will serve as a useful guide to the solution of the practical problems associated with separating and purifying fermentation and biotechnology products.

I would like to acknowledge the helpful suggestions of Henry C. Vogel, the patience of George Narita and the support and understanding of my wife, Joan Dechow. I also wish to recognize the assistance of Audrey Wildeck for secretarial support and of Bart Alazio for preparing the illustrations.

April 1989 Frederick J. Dechow

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1

Introduction

The recovery of products from fermentation or biochemical processes has been cited (1) as the last hurdle to be overcome in bringing biotechnology from the laboratory to commercial status. This text will attempt to describe how adsorptive materials, ion exchange resins, column chromatography and affinity chromatography can be utilized in these recovery and purification operations. This chapter will examine the nature of the fermentation broth and will serve to put these recovery operations in perspective with other purification techniques not covered by this text. It is essential to understand the relative advantages of each and their interrelationships since most purifications will require combinations of different techniques.

1.1 FERMENTATION BROTH

The fermentation broth is the combination of insoluble, gelatinous biomass, the nutrient fluid, and the soluble metabolites resulting from the fermentation operation. When the fermentation is carried out without any form of inert support for the biomass, the limit of fluidity for stirring or aeration is approximately 3 to 7% wv dry weight of biomass. Physically, biomass is a compressible, gelatinous solid with surface layers of polysaccharide material which make it cohere and adhere. Downstream processing, therefore, has to deal with a viscous, highly non-Newtonian slurry as its feedstock.

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For example, a bacterial fermentation for single cell protein will produce a broth of 3% wv in which the slurry is about 60% (by volume) wet biomass and 40% interparticle fluid. When biomass supports are used, somewhat higher operating biomass concentrations are possible and, in waste disposal fermentations, the concentration has been raised from 2 to 5 g/liter to 10 to 40 g/liter.

Compared with the feed streams to recovery processes in conventional chemical processing, fermentation broths are very dilute aqueous systems (see Table 1.1). Therefore, it will be particularly important to avoid energy intensive thermal operations and to select processes which give large concentration increases in the first stage or stages.

Table 1.1: Typical Product Concentrations Leaving Fermenters

Product	Grams per Liter
Antibiotics (e.g., Penicillin G)	10-30
Enzyme protein (serum protease)	2-5
Ethanol	70-120
Lipids	10-30
Organic acids (citric, lactic)	40-100
Riboflavin	10-15
Vitamin B12	0.02

The fluid volume in microbiological processes must be reduced by at least an order of magnitude between the broth and the final fluid stages of the recovery processes and, in some cases, by very much more. For vitamin B-12 the reduction ratio is over 1000:1. Consequently, the plant design and the range of acceptable unit operations changes significantly as the fluid progresses from the broth handling stages to the final isolation stage.

Many fermentation broths are unstable. Once any broth leaves the controlled, aseptic environment of the fermenter, it is exposed to a drastic change of conditions. An actively growing biomass from an aerated culture will be suddenly deprived of oxygen and will experience a rapidly falling concentration of nutrients. This frequently produces rapid changes in physical properties, leading to destruction of desired product. Lipids may be consumed as an alternative energy source for continuing metabolic activity. Enzymes may be destroyed by proteases released from the deteriorating cells. The broth also becomes susceptible to

contamination from foreign organisms which can have the same effects.

Similar problems can occur if the recovery operations of a batch fermentation are delayed. The problems can be reduced by chilling to around 5°C. This is commonly done for enzymes and other relatively small output processes. However, this is to be avoided, if possible, with larger fluid volumes since chilling from a typical fermentation temperature of 35°C to 5°C requires refrigeration energy of about 40 kWh/m^3 of broth and considerable capital expenditure. The time for appreciable product loss to occur can be as little as 20 minutes at fermentation temperatures.

The necessary and practical recovery operations employed and the order in which they are used in downstream processing can be reduced to deceptively simple looking recovery sequences. Figure 1.1 (2) shows a schematic of the recovery sequence and the techniques associated with each process.

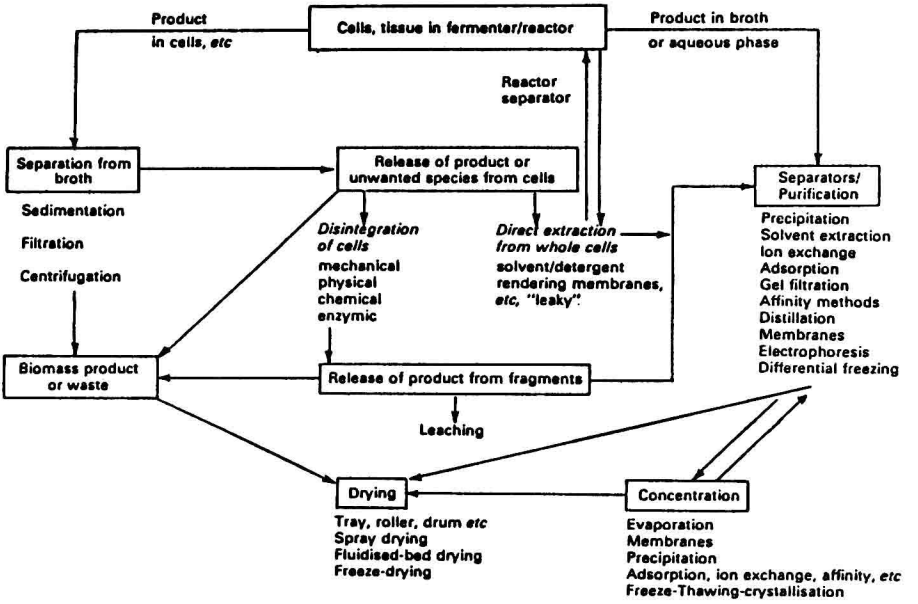

Figure 1.1. Schematic of the processes which may be involved in the separation and fractionation of fermentation products (Reference 2).

If the desired product is extracellular, it is only necessary to filter the biomass from the broth and isolate the product from the fluid. If a product is intracellular, a cell disruption step must first be employed. If the product is also water soluble, this disruption should be performed while the biomass is still in a slurry form. The chemical stability and solubility of the product will dictate the most suitable recovery techniques. Most microbiological products have limited chemical stability. This puts severe restrictions on the temperatures, the reactants, and pH levels which can be used.

An important consideration in determining the appropriateness of a recovery technique is the actual purity requirement for the product. Many products, such as enzymes and vaccines, will not need to be isolated as pure compounds. An appropriate product in these cases would be a complex mixture having the desired properties. However, removal of specific materials, such as pyrogens in injectable medicinals, would be necessary.

The major recovery difficulties arise when it is necessary to separate specific compounds from other chemically and physically similar materials. The isolation of enhanced purity enzyme protein from other protein requires highly specific physico-chemical effects to be used. Today it is the exception rather than the norm for the separated compounds to be that similar. Normally there is a range of alternative recovery techniques and a selection can be made on the basis of cost, familiarity, and reliability criteria.

1.2 RECOVERY UNIT OPERATIONS

The various methods for treating the fermentation broth can be divided into mechanical or chemical unit operations. Table 1.2 lists the processes normally included in each category.

Filtration and centrifugation are unit operations in which the suspended solids are separated from the fluid phase. Drying is the removal of moisture or solvent from solid particles, while evaporation is the removal of moisture or solvent from a solution. In crystallization, the conditions of a solution are adjusted to change the solubility of one of

Table 1.2: Separation and Recovery Techniques

Mechanical	Chemical
Filtration	Adsorption
Centrifugation	Ion exchange
Evaporation	Column chromatography
Crystallization	Affinity chromatography
Drying	Solvent extraction
Reverse osmosis	Electrophoresis
Ultrafiltration	Electrodialysis

the dissolved compounds so that it leaves the solution as a solid.

Microfiltration, ultrafiltration, and reverse osmosis are membrane processes in which separation is based on differences in ability to flow through a thin barrier that separates two fluids. Microfiltration is a hydraulically driven process using a membrane with a pore size in the 100 to 3000 Å range. For ultrafiltration, the pore size is from 10 to 125 Å, while for reverse osmosis, the pore size is from 3 to 10 Å.

Adsorption, ion exchange, column chromatography, and affinity chromatography can be grouped as recovery techniques in which the removed compound or solute establishes an equilibrium between sites on a solid phase material and the solution. In adsorption, the removed species is bonded to the solid phase material by polarity or weak chemical bonds. Ion exchange recovers material by the interchange of ions between the liquid and solid phases. Column chromatography may use adsorptive, ion exchange or molecular sieve materials to separate solutes which are first loaded onto a column of the separation material and then eluted in such a manner that the individual solutes are collected in separate fractions. In affinity chromatography, the removed species is bound with a high level of selectivity to ligands covalently attached to a solid matrix.

In solvent extraction, the removed compound establishes an equilibrium distribution between immiscible solvents, usually water and an organic liquid.

Electrophoresis and electrodialysis are separation techniques that separate charged molecules or ions using an

electric field. Electrophoresis separates charged components by accentuating small differences in ionic mobility in an electric field using a moving carrier fluid. Electrodialysis concentrates components on the basis of electromigration through ionic membranes.

1.2.1 Mechanical Operations

1.2.1.1 Filtration: Filtration is typically the first step in the isolation of any product from the fermentation broth. This process separates the biomass cells, the cell debris, and any precipitates from the broth fluid. The mathematical representation of the incremental time dt to filter an additional incremental volume dV after a volume V has been filtered is given by:

$$dt/dV = a + bV \qquad (1.1)$$

The right side of the equation contains two components, a and b. The a term is $\eta r_s/AP$ and the b term, which depends on V, is equal to $\eta r_c/A^2PW$, where η is the liquid viscosity, r_s is the specific cake resistance of the filter material, A is the filter area, P is the constant applied pressure difference, r_c is the specific cake resistance, and W is the cake dry weight per unit volume of filtrate. Normally the resistance of the filter material term includes the resistance contribution of any filtration aid, pipes, and valves.

According to this equation, the resistance to filtration is due initially to only the constant term a. In theory, as filtration proceeds and the biomass cake becomes thicker, the resistance would be expected to increase linearly according to the b term with this dependence on V. Although many practical considerations must be taken into account when applying this equation, its simplicity, the complexity of a more exact description, and the uniqueness of many industrial applications result in Equation 1.1 being the most useful filtration representation.

The specific practical limitations that must be considered are the blockage or blinding of the filter, the compressibility of the biomass cake, and the variable pore structure of the cake. Blinding of the filter may be prevented by starting the filtration at a low hydraulic pressure by partially by-passing the pump. This will avoid

driving the first solids into the filter support. The biomass cake's compressibility will usually be proportional to the applied hydraulic pressure up to a certain pressure. Beyond that pressure, the cake will collapse to a new compressed form so that throughput is reduced with the incremental pressure increase.

Table 1.3 shows filtration design and operation for different fermentation broths. In two of the cases noted in Table 1.3 a precoat was used. The filter will often be precoated with a filtration aid such as diatomaceous earth to reduce blinding of the filter and to increase filtration rates. The filtration aid might be added to the broth but then the quantity of filtration aid required is more than double the precoat amount (3).

Table 1.3: Representative Design and Operating Results for Fermentation Broth (Vacuum 0.68–0.85 Bar)

	Bacillus licheniformis	*Streptomyces erythreus*	*Penicillium chrysogenum*
Filter type	Vacuum precoat	Vacuum drum	Vacuum drum or precoat
Design filtration rate (ℓ/hr-m^2)	160–320	400	1,400–1,800
Solids in slurry (wt %)	8	25	2–8
Cycle time (min/rev)	0.5	3	8
Filter medium	Precoat	Nylon	Polypropylene
Cake discharge mechanism	Precoat	String	String or precoat

The equipment in this operation can be as simple as an enlarged laboratory vacuum filter to more elaborate rotary vacuum filters. These latter filters essentially consist of a hollow segmented drum covered with a filter cloth. The drum rotates in a bath of the broth to be filtered while a vacuum inside the drum sucks liquid through the filter cloth, forming a coating of solids on the outside of the drum. Provisions are usually made to wash the filter cake during filtration followed by removing the solid from the cloth. Instead of vacuum, pressure can be used to drive the fluid through a filter cake. Plate and frame pressure filters consist of wire or perforated metal frames which act as the mechanical support for the filter medium which can be fine

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