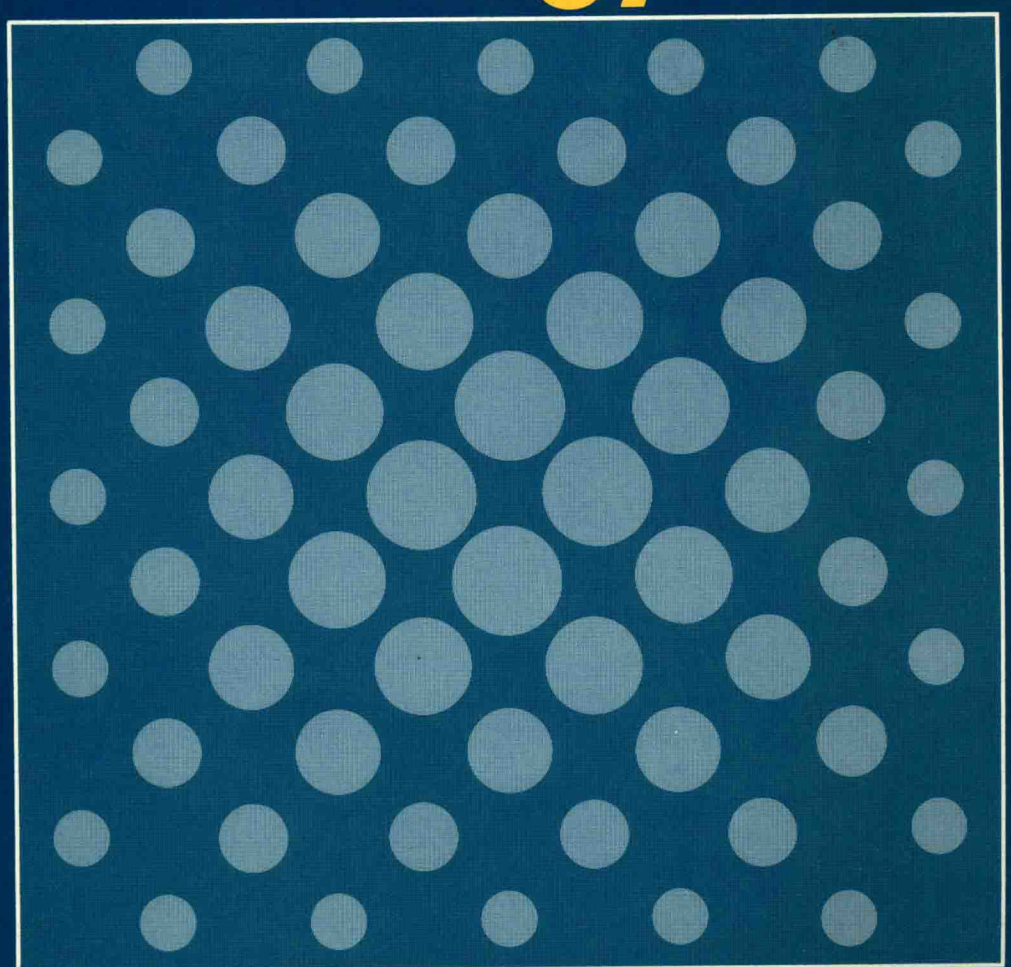
BIOTOL

BIOTECHNOLOGY BY OPEN LEARNING

Product Recovery in Bioprocess Technology



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The Biotol Project

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This series of books has been developed through a collaboration between the Open universiteit of the Netherlands and Thames Polytechnic to provide a whole library of advanced level flexible learning materials including books, computer and video programmes. The series will be of particular value to those working in the chemical, pharmaceutical, health care, food and drinks, agriculture, and environmental, manufacturing and service industries. These industries will be increasingly faced with training problems as the use of biologically based techniques replaces or enhances chemical ones or indeed allows the development of products previously impossible.

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Project Manager: Dr J. W. James

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An open learning text presents to you a very carefully thought out programme of study to achieve stated learning objectives, just as a lecturer does. Rather than just listening to a lecture once, and trying to make notes at the same time, you can with a BIOTOL text study it at your own pace, go back over bits you are unsure about and study wherever you choose. Of great importance are the self assessment questions (SAQs) which challenge your understanding and progress and the responses which provide some help if you have had difficulty. These SAQs are carefully thought out to check that you are indeed achieving the set objectives and therefore are a very important part of your study. Every so often in the text you will find the symbol Π , our open door to learning, which indicates an activity for you to do. You will probably find that this participation is a great help to learning so it is important not to skip it.

Whilst you can, as a open learner, study where and when you want, do try to find a place where you can work without disturbance. Most students aim to study a certain number of hours each day or each weekend. If you decide to study for several hours at once, take short breaks of five to ten minutes regularly as it helps to maintain a higher level of overall concentration.

Before you begin a detailed reading of the text, familiarise yourself with the general layout of the material. Have a look at the contents of the various chapters and flip through the pages to get a general impression of the way the subject is dealt with. Forget the old taboo of not writing in books. There is room for your comments, notes and answers; use it and make the book your own personal study record for future revision and reference.

At intervals you will find a summary and list of objectives. The summary will emphasise the important points covered by the material that you have read and the objectives will give you a check list of the things you should then be able to achieve. There are notes in the left hand margin, to help orientate you and emphasise new and important messages.

BIOTOL will be used by universities, polytechnics and colleges as well as industrial training organisations and professional bodies. The texts will form a basis for flexible courses of all types leading to certificates, diplomas and degrees often through credit accumulation and transfer arrangements. In future there will be additional resources available including videos and computer based training programmes.

Preface

The remarkable advances in biotechnology in the past few decades can be attributed to a combination of increased knowledge of how biological systems function and on developments in the process technology associated with production and harvesting of the products of biotransformations. Central to the successful application of the opportunities arising from the new knowledge of biological phenomena are the issues of bioproduct recovery. This text is about the recovery of bioproducts.

The recovery of the desired products from bioreactor outflow is greatly influenced by the nature of the products themselves, the size of the market, the need to achieve market and legally required specification standards and on the market value of the product. The achievement of preparing a product with desired characteristics in a commercially viable manner is, therefore, not a simple matter. It not only requires knowledge of the technical and scientific principles involved in product recovery, but that selection of appropriate strategies be made to achieve commercial and specification targets. This texts aims to provide the knowledge needed to understand, select and develop strategies for the recovery of products from bioreactors and to develop awareness of the issues relating to the final formulation of products. This experience will enable readers to contribute to the commercial realisation of bioproduct manufacture.

We have been fortunate with our author:editor team in bringing together both real life industrial experience of the key processes and experience of sound teaching and learning principles. This combination has prepared a text which not only reflects the forefront of commercial downstream processing, but which also provides easy to read, sound instruction in the strategies and techniques employed in this vitally important aspect of bioprocess technology. To achieve this, in-text activities have been incorporated into the text to facilitate learning and readers are provided with opportunities to check their own learning through the inclusion of self assessment questions.

The text begins by putting downstream processing into a commercial context. It then goes on to examine the starting point of downstream processing, the fermentation broth. Consideration is then given of the processes available for the release of intracellular components and the primary processes available for separating solids from solid-liquid suspensions. Subsequent chapters deal with concentrating and purification of products. Product formulation and market research are essential for the successful commercialisation of biotechnology. These topics are dealt with in Chapter 8. The final chapter deals with the more recently introduced separation processes and discusses the integration of downstream and upstream processes. The text is supported by appendices which provide a summary of the symbols used within the text and useful physical constants. Suggestions for further reading are also included.

Scientific and Course Advisors: Dr M. C. E. van Dam-Mieras
Dr C. K. Leach

Contents

Downstream processing in biotechnology

Downstream processing in biotechnology

1.1 Introduction

Central in biotechnology are living cells making desired (and often sophisticated) products. These are formed in a complicated and often quite dilute mixture in a bioreactor. 'Downstream' from this reactor we then have to concentrate and purify the desired product. Downstream processing is the principle subject of this book.

economic product recovery

Downstream processing is not the most glamorous part of biotechnology. A glimpse in any introductory book on the subject will show you that. Chances are that it will not take up more than a few percent of the text. This can be understood - at least partly. Nowadays we know infinitely more about microbiology, genetics and biochemistry than we did a few decennia ago. This has opened our eyes to the possibilities for making products and applications which were formerly undreamt of. The development of these new applications always starts in the laboratory using small scale analytical and preparative techniques. However, in the further development of biotechnological products processing usually becomes very important. As the scale increases, economic ways of conducting fermentation and product recovery become essential. Production scales are larger and changes in the process are more difficult to implement. In this stage downstream processing may require half or more of the cost price. It is therefore worthwhile to try to conceive a proper downstream scheme earlier in the process development. This is difficult: it requires that not only process engineers, but also laboratory development personnel have some idea of which steps are economical and easily scaled up. On the other hand it must be said that small improvements on a production scale can greatly improve the economics of a process.

1.2 Different sectors in biotechnology

In biotechnology, the concept of recovery and product purification is different for the different market sectors. To those manufacturers trying to develop a new drug (a therapeutic enzyme, for example), recovery and purification are just laboratory techniques, only to be used on a preparatory scale. In the production of bulk antibiotics and enzymes, where a high stage yield and a low cost price are very important, a typical chemical engineering approach is more common.

market volume and selling price

In biotechnology we may distinguish a number of market sectors based on market volume and selling price.

In Figure 1.1 the selling price is plotted as function of the concentration in the starting material (blood plasma, fermentation broth, etc).

The products in the upper left corner in particular look very attractive. Selling prices up to 10^6 US $ kg^{-1} strongly appeal to one's imagination. The total market volume however is rather low. The world demand for factor VIII, for example, is about 0.1-1 kg a year. The world market for penicillin is $1.5\ 10^7$ kg a year with a total turnover of 4.10^8 US $ a year, equalling the total turnover of factor VIII.

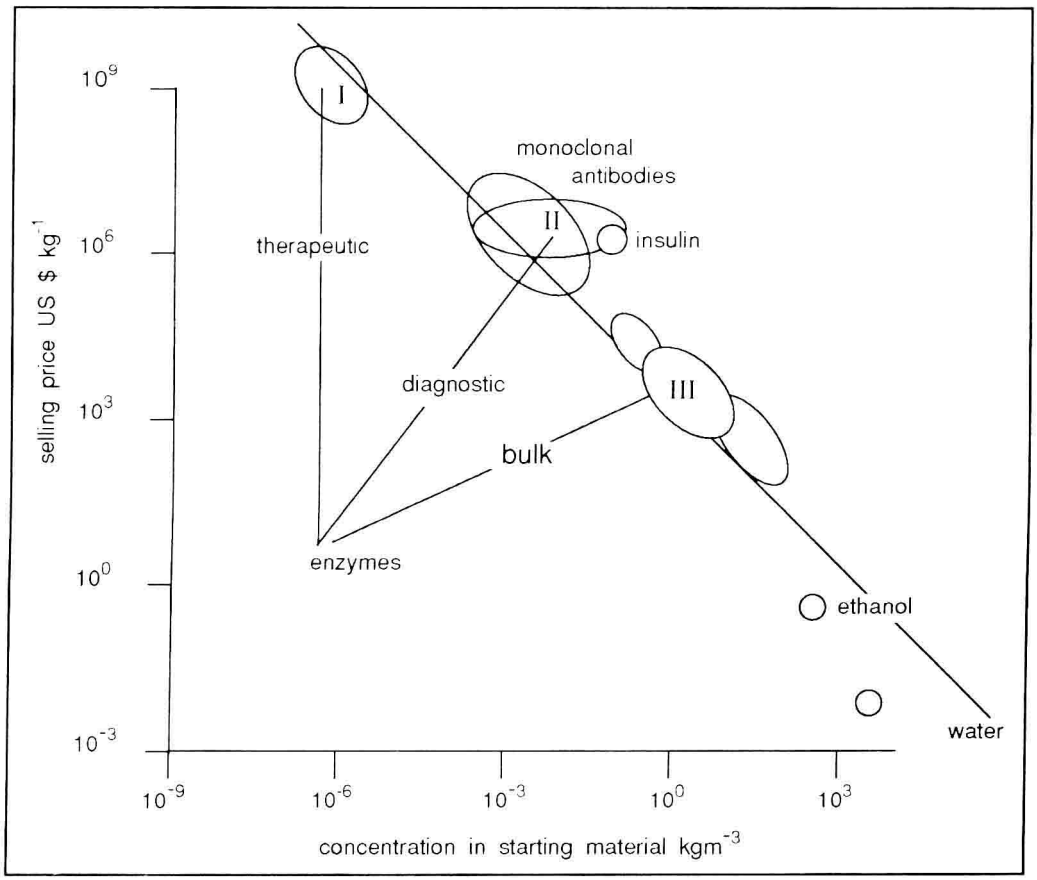

Figure 1.1 Selling price of bioproducts as function of the concentration in starting material.

added value influences net profit

It would seem that the product of selling price and market volume for the different products is roughly constant. However, the added value and, consequently, the net profit may differ greatly.

three market sectors

Three different market sectors can be distinguished:

- Sector I can be characterised as the therapeutic protein sector, with products such as factor VIII and urokinase.
- Sector II is the area of research into diagnostic enzymes and monoclonal antibodies. Other products in this sector are: insulin, luciferase and glycerophosphate dehydrogenase.
- Sector III is characterised by industrial bio-bulk products ranging from antibiotics, proteases, amylases and organic acids to ethanol.

Table 1.1 gives some typical characteristics of bioprocesses in the different sectors.

As can be seen from the table, downstream processing may differ quite substantially from one sector to the other.

Characteristics	Sector I	Sector II	Sector III
Volumes	0.1-10^2 kg y^{-1}	10^3-10^5 kg y^{-1}	10^6 - 10^9 kg y^{-1}
Organism	recombinant-DNA	partly recombinant-DNA	natural producers
Product purity	very high	high/very high	relatively low
Recovery yield	subordinate importance	of minor importance	highly important
Cost price	fraction	20-50% determined by raw materials	50-90% determined by raw materials
Technology	affinity chromatography, preparative electrophoresis	adsorption chromatography, membranes	filtration / extraction / adsorption / precipitation / evaporation / membranes

Table 1.1 Characteristics of bioprocesses in market sectors.

Many of the products in sectors I and II are manufactured under Good Manufacturing Practice (GMP). The process is carefully designed and validated according to the requirements of the national authorities or the Food and Drug Administration (FDA). But in these sectors many processes are being run under suboptimal conditions because early product registration and the wish to be the first on the market are more important than optimal processing.

Many of the processes in sector III are not restricted by these stringent rules and in this field the typical chemical engineering approach works very well.

SAQ 1.1

	Enzyme A	Enzyme B
Selling price (US$ kg^{-1})	5 x 10^4	100
Total market volume (kg y^{-1})	5 x 10^2	2 x 10^6
Added value (US$ kg^{-1})	12 x 10^3	10

1) Determine the total turnover for each enzyme.

2) Determine the annual net profit for each enzyme.

3) To which market sector do each of these enzymes belong?

4) Which of these enzymes is likely to have the highest concentration in the starting material (eg fermentation broth)? Give a reason for your choice.

1.3 Characterisation of biomolecules

Because biomolecules differ greatly in nature, different separation principles are required for their recovery and purification.

biomolecules as products

Their relative molecular masses vary from approximately 60 to over 2,000,000. Generally, biomolecules are rather unstable and their stability depends on many different factors such as:

- pH;
- temperature;
- ionic strength;
- type of solvent used;
- presence of surfactant;
- metal ions, etc.

In addition, many biomolecules are sensitive to shear and are hydrophobic. Finally, they are sometimes present in very low concentrations, as can be seen in Figure 1.1. These characteristics of biomolecules strongly influence the characteristics of bioprocesses used for their production.

1.4 Characterisation of bioprocesses

One of the most striking characteristics of bioprocesses is the difference in production scale. As can be seen from Table 1.1, the difference is a factor 10^{10}.

Table 1.2 shows some typical characteristics of bioprocesses.

- almost exclusively batch
- small scale relative to chemical industry
- multifunctional equipment
- very flexible and easy to extend
- equipment sterilisable
- suited for containment production
- validated equipment in case of pharmaceutical production

Table 1.2 Characteristics of bioprocesses.

recovery processes should be flexible and easy to extend

Recovery processes in particular should be easy to extend. When starting a new product, generally the expression levels are very low. During production, the fermentation process will be optimised, and medium and strain selection will continue. The result is a strong increase in the expression level. A classical example is penicillin.

In forty years the concentration has increased from 0.4 to approximately 80 mol m^{-3}, an increase of a factor 200! The process should be sufficiently flexible to handle strong fluctuations in fermentation behaviour. These fluctuations are the result of changes in raw materials or strains used during the fermentation. Also, the behaviour patterns of micro-organisms may be fairly unpredictable.

1.5 Recovery in modern versus classical biotechnology

low number of unit operations and high purity

Modern biotechnology is characterised by the way in which organisms are conditioned to make their products. In modern biotechnology, products are made by organisms that do not produce these by nature, but have been manipulated by recombinant DNA (rDNA) techniques in order to achieve this.

The recovery is restricted to a limited number of process steps of high resolution. The degree of purification is high to very high.

Typical characteristics of modern biotechnology processes are given in Table 1.3.

- production on a small scale, 0.1-10m^3
- extension of the variety of organisms used for production (plant and mammalian cells)
- use of non classical fermenters such as:
  - airlift;
  - membrane reactor;
  - immobilised cell reactor.
- sometimes continuous or equipped with *in situ* recovery system

Table 1.3 Characteristics of contemporary bioprocesses.

The separation of biomolecules is largely focused on biospecific interactions and properties such as:

- molecular weight (mass);
- charge distribution;
- hydrophobicity;
- immunogenic structure;
- structure.

In classical biotechnology, however, the product is made by the natural producers (molds for penicillin, bacteria for organic acids and proteases, etc).

high number of unit operations

In many cases a large sequence of classical unit operations of low resolution is necessary to achieve the required purity.

Most of the unit operations are relatively well described mathematically and scale up parameters are relatively easy to determine in pilot plant experiments.

The yield is an important factor, as is minimising the costs of raw materials and labour.