OPEN UNIVERSITY PRESS BIOTECHNOLOGY SERIES

Bioprocessing

Owen P Ward

In association with the Institute of Biology IoB

Bioprocessing

Owen P. Ward

Open University Press
Milton Keynes

Open University Press
Celtic Court
22 Ballmoor
Buckingham
MK18 1XW

First published 1991

British Library Cataloguing-in-Publication Data

Ward, Owen P.
Bioprocessing. – (Biotechnology series)
I. Title II. Series
660

ISBN 0–335–09363–9

Typeset by Vision Typesetting, Manchester
Printed in Great Britain by Biddles Limited, Guildford and King's Lynn

Bioprocessing

The Biotechnology Series

This series is designed to give undergraduates, graduates and practising scientists access to the many related disciplines in this fast developing area. It provides understanding both of the basic principles and of the industrial applications of biotechnology. By covering individual subjects in separate volumes a thorough and straightforward introduction to each field is provided for people of differing backgrounds.

Titles in the Series

Biotechnology: The Biological Principles: M.D. Trevan, S. Boffey, K.H. Goulding and P. Stanbury
Fermentation Kinetics and Modelling: C.G. Sinclair and B. Kristiansen (Ed. J.D. Bu'Lock)
Enzyme Technology: P. Gacesa and J. Hubble
Animal Cell Technology: M. Butler
Fermentation Biotechnology: O.P. Ward
Genetic Transformation in Plants: R. Walden
Plant Biotechnology in Agriculture: K. Lindsey and M.G.K. Jones
Biosensors: E. Hall
Biotechnology of Biomass Conversion: M. Wayman and S. Parekh
Process Engineering in Biotechnology: A.T. Jackson
Biotechnology in the Food Industry: M.P. Tombs
Plant Cell and Tissue Culture: A. Stafford and G. Warren (eds.)
Bioprocessing: O.P. Ward

Series Editors

Professor J.A. Bryant — *Department of Biology, Exeter University, England*
Professor J.F. Kennedy — *Department of Chemistry, Birmingham University, England*

Series Advisers

Professor C.H. Self — *Department of Clinical Biochemistry, University of Newcastle upon Tyne, England*
Dr R.N. Greenshields — *G.B. Biotechnology, Swansea, Wales*

The Institute of Biology IOB

*This series has been editorially approved by the **Institute of Biology** in London. The Institute is the professional body representing biologists in the UK. It sets standards, promotes education and training, conducts examinations, organizes local and national meetings, and publishes the journals **Biologist** and **Journal of Biological Education.***

For details about Institute membership write to: Institute of Biology, 20 Queensberry Place, London SW7 2DZ.

Preface

Methods for processing of biological materials into useful products represent essential core manufacturing activities of the food, chemical and pharmaceutical industries. On the one hand the techniques involved include well established process engineering methodologies such as mixing, heat transfer, size modification and a variety of separation and fermentation procedures. In addition, new bioprocessing practices arising from the exciting recent advances in biotechnology, including innovative fermentation cell culture and enzyme based operations, are rapidly extending the frontiers of bioprocessing. These developments are resulting in the introduction to the market place of an awesome range of novel biological products having unique applications. Indeed, the United States Office of Technology Assessment* has concluded that 'competitive advantage in areas related to biotechnology may depend as much on developments in bioprocess engineering as on innovations in genetics, immunology and other areas of basic science'. Advances in analytical instrumentation, computerization and process automation are playing an important role in process control and optimization and in the maintenance of product quality and consistency characteristics. Bioprocessing represents the industrial practice of biotechnology and is multidisciplinary in nature, integrating the biological, chemical and engineering sciences. This book discusses the individual unit operations involved and describes a wide variety of important industrial bioprocesses.

I am very grateful to Sanjay Thakur who assisted me in the collection of material for this book. A very special acknowledgement is due to my colleague, Val Butler, who played a major role in production of the manuscript, including word processing and layout of text and figure and table design.

Owen Ward

* Commercial Biotechnology: An International Analysis (Washington D.C. US Congress, Office of Technology Assessment, OTA-BA-218, 1984).

Acknowledgements

I wish to thank the following publishers and organisations for granting permission to reproduce original or copyright material.

Biotage Inc., Charlottesville, VA: Figure 4.5.

Bio/Technology, New York. Figure 8.9 from Posillico, E.G. (1986). Microencapsulation technology for large-scale antibody production, *Bio/Technology* **4**, 114–117.

Butterworths Heinemann, US: Table 10.4 from Dordick, J.S. (1989). Enzyme catalysis in monophasic organic solvents. *Enzyme and Microbial Technology* **11**, 194–211.

CRC Press, Boca Raton, Fl: Table 5.1 from Chichester, D.F. and Tanner, F.W. (1972). Antimicrobial Food Additives. In *Handbook of Food Additives*, Ed. Furia, T.E. pp. 115–184.

Dekker, New York: Figure 8.7 from Seaver, S.S. (1987). Culture method affects antibody secretion of hybridoma cells. In *Commercial Production of Monoclonal Antibodies*, Ed. Seaver, S.S. pp. 93–118. Figure 8.8 from Lebherz III, W.B. (1987). Batch production of monoclonal antibodies in large-scale suspension culture. In *Commercial Production of Monoclonal Antibodies*, Ed. Seaver, S.S. pp. 93–118. Figure 8.10 from Von Wedel, R.J. (1987). Mass culture of mouse and human hybridoma cells in hollow-fibre culture. In *Commercial Production of Monoclonal Antibodies*, Ed. Seaver, S.S. pp. 159–173.

Ellis Horward, Chichester: Table 3.1 from Kennedy, J.F. and White, C.A. (1985). Principles of immobilization of enzymes. In *Handbook of Enzyme Biotechnology*, 2nd ed., Ed. Wiseman, A. pp. 147–207.

Society for General Microbiology: Figure 4.8 from Lambe, C.A. (1986). Continuous electrophoresis for production-scale purification. In *Bioactive Microbial Products 3: Downstream Processing*, Ed. Stowell, J.D., Bailey, P.J. and

Winstanley, P.J. pp. 191–203. London Academic. Figure 7.6 from Low, D.K.T. (1986) Chromatographic methods. In *Bioactive Microbial Products 3: Downstream Processing*, Ed. Stowell, J.D., Bailey, P.J. and Winstanley, P.J. pp. 121–145. London Academic. Figure 8.6 from Atkinson, T., Barstow, D.A., Court, J., Minton, M.P., Sharp, R.J. and Sherwood, R. (1986). High level microbial expression and purification of recombinant proteins. In *Bioactive Microbial Products 3: Downstream Processing*, Ed. Stowell, J.D., Bailey, P.J. and Winstanley, P.J. pp. 27–43. London Academic.

Wiley, New York: Figure 4.9 from Snowman, J.W. (1988). Lyophilization techniques, equipment and practice. In *Downstream Processing, Equipment and Techniques*. Ed. Mizrahi, A., pp. 315–351. New York, A.R. Liss. Figure 8.4 from Ryu, D.D.Y. and Hospodka, J. (1980). Quantitative physiology of *Penicillium chryosogenum* in penicillin fermentation. *Biotechnol. Bioeng.* **20**, 289–298.

Contents

Chapter 1

Introduction: Biomaterials and Bioprocessing

The processing of biological materials into useful products has been practised throughout the ages. Because of the dependence of mankind on food for survival, methods of preservation of agricultural produce to facilitate food storage have always been of prime concern. In addition, there has always been a need or desire to modify basic agricultural produce to separate out the digestible material and to improve food quality, especially with respect to flavour, taste and general palatability. Early processing methods reflected the availability and development of processing implements made from wood, stone, metal and other materials. Particularly in the last century, engineering aspects of bioprocessing have benefitted from developments in mechanization and the continual development of new equipment and synthetic processing materials. In addition, sophisticated automation and control methodologies arising from advances in electronics and computing are benefitting bioprocess engineering.

An important aspect of modern bioprocessing methodology is the use of cell or enzyme systems to produce new substances or to catalyse reactions modifying natural or synthetic materials. Indeed, micro-organisms and enzymes were used for many centuries before their existence was known. Although scientists first concluded that micro-organisms are 'living things' around 1836, and Pasteur concluded that yeast cells could convert sugar to ethanol in 1856–7, records of alcoholic beverages made by the fermentation of grape or cereal extracts, the use of mouldy bread for wound healing, and the curdling of milk carried in calf stomachs all date back to ancient times (Rose, 1981). The mouldy bread probably contained antibiotics, and calf stomachs contain the cheese-clotting enzyme, rennet. Following Pasteur's observations, pure-culture microbiological methods were established, ultimately leading to the development of microbial fermentation processes for the production of enzymes, chemicals and pharmaceuticals, and improved methods for the manufacture of fermented foods. The introduction of

industrial enzymes led to the development of enzyme applications in food processing, in biotransformation of chemicals and pharmaceuticals, and in other areas of bioprocessing. Microbiological methods were applied to the cultivation of animal cells for viral vaccine production, and plant cell culture techniques were used primarily for the purpose of plant breeding.

Major advances in the development of genetic techniques such as mutation and cell fusion were made during the golden antibiotic era which followed the pioneering research on penicillin by eminent scientists such as Fleming, Florey and Chain. Applications of classical genetics combined with improvements in fermentation technology increased the amount of penicillin produced per litre of culture from a few milligrams per litre to 20 g l^{-1} (Aharonowitz and Cohen, 1981). More recently, the introduction of recombinant DNA technology has given bioprocessing a new impetus, facilitating the construction of cell systems capable of synthesis of heterologous or foreign proteins (for example, synthesis of human proteins in microbial systems) and the capacity to engineer totally novel proteins. In addition, this technology makes it possible to synthesize specific proteins in extremely high yields. The development of hybridoma technology (Kohler and Milstein, 1977) for the production of monoclonal antibodies has led to major advances in animal cell culture techniques in response to the huge demand for monoclonal antibodies for analytical and therapeutic applications.

In the longer term, techniques of molecular and cellular biology – combined with more classical scientific methodologies – offer much potential for the breeding of new varieties of plants and animals which have improved nutritional composition and productivity, or which are more amenable to bioprocessing methods or are capable of synthesizing novel products.

Important milestones relating to the applications of cell culture, enzymology and molecular biology to bioprocessing are summarized in Table 1.1.

The physical properties and chemical composition of biomaterials and also, where relevant, bioactivity, determine the applications of these materials, the nature of products which may be produced and the processing methodologies. The effective use of cell and enzyme systems in bioprocessing production methods requires an understanding of microbial physiology and biochemistry and a capacity to develop conditions which optimize biosynthesis or bioconversion procedures.

1.1 Raw materials

The general composition of key constituents in selected raw materials is presented in Table 1.2. The cereals, which have a moisture content of 10–15%, are major sources of carbohydrates (67–77%). While tubers contain 70–80% water, they also represent a major carbohydrate source (75–95%) on a dry-weight basis. Nuts and seeds have variable moisture contents, and many contain greater than 50% by weight of lipid material. Legumes are characterized by high levels of protein in combination with a substantial carbohydrate content. The unusually high protein content of soya bean (35%) makes it an important source of nutritional protein.

Table 1.1 Milestones in the use of cell culture, enzymology and molecular biology in bioprocessing

1837–8	Conclusion that yeast is a living organism
1856–60	Conclusion that living yeast converts sugar to ethanol and CO_2 First microscopic observation of yeast
1881	Development of pure culture and other classical bacteriological methods Commercial production of lactic acid by fermentation
1885–90	Development of the first cholera, diphtheria and tetanus vaccines
1910	Development of classical mammalian cell culture techniques
1917	Development of industrial fermentation for the production of amylases and diastases by surface culture
1923	Commercial production of citric acid by fermentation
1928	Observations of the antagonistic effect of filtrates of *Penicillium notatum* on *Staphylococcus aureus*
1936	Discovery that infective tubercle bacilli could be serially subcultured to produce attenuated (non-disease-causing) vaccines
1940–1	Isolation of penicillin and development of commercial fermentation process
1952	Discovery of capacity of *Rhizopus arrhizus* to convert progesterone to 11 α-hydroxyprogesterone
1973	Cloning of first gene
1974	First expression of a gene from a different species in bacteria
1975	Hybridoma created for first time
1981	Monoclonal antibody diagnostic kit approved for use in US
1982	rDNA animal vaccine (for colibacillosis) approved for use in Europe Human insulin produced by rDNA approved for use in US
1983	First plant gene expressed in a different species
1985	Commercial production of shikonin from plant cell culture Production of human growth hormone in transgenic animals
1986	First approval of a genetically engineered human vaccine – yeast produced hepatitis B subunit vaccine
1987	Recombinant tissue plasminogen activator approved by the US FDA US approval given for Phase 1 clinical trials on a recombinant AIDS vaccine
1988	Production of transgenic soybean plants First patent on an engineered enzyme issued – subtilisin with amino acid substitutions
1989	Recombinant erythropoietin approved for use in treating anaemia associated with chronic renal failure
1990	Wide range of recombinant products in various countries at different stages of clinical testing and development, including: anticoagulants, thrombolytic agents, colony stimulating factors, growth hormones, tumour necrosis factors, interferons, interleukins, monoclonal and chimeric antibodies. First bioengineered food additive, chymosin for cheese-making, produced in *E. coli*, approved in the US

Table 1.2 Composition of selected raw materials used in bioprocessing*

	Water	*Protein*	*Lipid*	*Carbo-hydrate*
Cereals				
Barley	14.0	7.4	1.3	76.6
Maize	14.5	8.6	5.0	70.6
Oats	11.5	13.5	5.6	67.6
Rye	13.5	8.5	1.6	75.0
Rice	15.5	9.2	1.3	73.4
Wheat	14.5	11.7	1.8	71.6
Tubers				
Artichoke	81.2	1.9	0.2	15.5
Potatoes	79.5	2.0	0.2	17.2
Nuts and seeds				
Brazil nuts	4.7	17.4	65.0	9.8
Chestnuts	60.2	2.7	0.3	35.5
Hazelnuts	4.7	12.7	58.8	21.1
Peanuts	6.2	25.4	47.4	19.8
Pecans	3.2	11.0	72.0	12.2
Legumes				
Broad beans	13.3	26.0	2.0	55.9
Kidney beans	16.5	19.9	2.2	57.8
Peas	13.4	21.7	2.3	60.4
Soybeans	12.5	35.3	19.0	28.2
Vegetables (raw)				
Asparagus	93.1	1.9	0.1	4.2
Broccoli	84.9	5.9	0.1	7.8
Cabbage	92.4	1.4	0.1	5.5
Carrots	90.4	1.2	0.2	7.0
Celery	95.3	0.9	0.1	2.8
Cucumber	96.2	1.0	0.2	2.0
Onion	90.4	1.0	0.1	8.1
Root turnip	94.7	0.9	0.1	3.7
Fruits (raw)				
Apples	85.8	0.2	0.1	13.6
Avocados	70.1	2.5	18.7	7.3
Bananas	75.0	1.1	0.1	22.9
Cherries	84.8	1.0	0.2	13.5
Grapefruit	89.6	0.8	0.1	9.1
Grape	84.4	0.5	0.2	14.6
Lemons	87.3	0.8	0.6	10.8
Melons	87.6	0.7	0.1	11.0
Oranges	86.8	0.9	0.1	11.8
Peaches	89.3	0.6	0.1	9.6
Pineapple	83.6	0.4	0.1	15.6
Strawberry	90.1	0.9	0.2	8.3
Tomatoes	95.0	0.7	0.1	3.7
Meats				
Broiler (thigh)	73.5	18.0	7.4	0.1
Cattle (sirloin)	51.0	16.9	31.0	0.3
Duck	54.3	16.0	28.6	0.1
Sheep	64.2	17.9	17.0	0.1
Swine	65.4	19.7	13.2	0.6
Turkey	72.9	19.6	6.5	0.1
Fish and shellfish				
Carp	75.4	17.3	6.0	0.2
Cod and pollack	82.7	15.7	0.4	0.0
Herring	65.3	16.0	17.0	0.1
Mackerel	62.5	19.8	16.5	0.1
Salmon	69.3	20.7	8.4	0.1
Scallop	81.2	13.8	1.2	1.8
Eggs				
Chickens'	74.7	12.3	11.2	0.9
Biological fluids				
Cows' milk	88.6	2.9	3.3	4.5
dried blood	–	81.0	0.8	–
Micro-organisms				
Brewer's yeast		43.0	1.5	39.5
Fusarium graminearum		42.0	13.0	ND
Micro-organisms in general		40–60	10–15	15–25

Wood materials	*Cellulose*	*Hemicellulose*	*Lignin*
Softwood	45–50	15–20	25–30
Hardwood	45–50	20–25	20–25

*Compiled from Sudo *et al.* (1989); Rechcigl (1982); Rose (1979); Anderson and Solomons (1984) and Miller and Churchill (1986).

Fruits and vegetables generally have moisture contents ranging from 75 to 96%, and a low lipid content. Avocado, which contains moisture and lipid contents of 70% and 19%, respectively, is an exception. Protein and carbohydrate levels tend to be variable. Meat and fish have moisture content ranges of 50–75% and 60–83%, respectively. For most meat and fish materials, the protein content ranges from 14 to 20%, and the lipid content is variable, ranging from 6 to 30% in meats and 1 to 21% in fish and shellfish.

The low moisture content of cereals allows these materials to be ground or milled in dried form as an initial processing method. Dry size reduction methods can be designed to facilitate separation of different cereal components following grinding based on physical property differences of the particulate components. Nevertheless, for the processing of cereals such as corn for the production of starch and other products, wet milling is better than dry milling as it facilitates subsequent separation of corn components (section 6.1). When cereals are used as a raw material for alcoholic fermentations, the ratio of water added to cereal is generally 3–5:1. In contrast, when the potato tuber is used as a raw material for alcoholic fermentations, starch gelatinization by cooking may be carried out without added water because of the high moisture content of these materials. Where vegetables, fruits, meat and fish, all of which have a high moisture content, are used as raw materials, initial processing stages may involve cutting, homogenization, pressing or extraction (see Chapter 6). In some cases, the materials may be initially dried as a means of preservation or to facilitate application of milling or grinding processing methodologies. In some bioprocesses, materials with a high moisture content or biological fluids are frozen, freeze dried or spray dried as an initial processing step. In general, intracellular products of cell culture are recovered following disruption of an aqueous cell suspension. Where the desired product is water insoluble, organic solvent extraction procedures are usually applied. Where the product exists in dilute form in the extracellular fluid, a product-concentration step is often necessary. Because of the physico-chemical properties of lignocellulose, wood generally requires more severe initial processing operations than do other biomaterials when it is used as a raw material for bioprocessing. Appropriate methodologies may include chemical pretreatment, high-temperature treatment or the production of fine grind powders.

Methods for bulk processing of biological materials are discussed in Chapter 2, and applications of these methods in the processing of animal and plant materials are illustrated using selected examples in Chapter 6.

1.2 Cell cultivation

A detailed discussion of cell physiology and biochemistry and the biological factors influencing microbial, mammalian and plant cell growth and product formation is beyond the scope of this book. Selected properties of organisms relevant to bioprocessing are set out in Table 1.3. In the following discussion some of the key factors will be briefly highlighted.

Table 1.3 Examples of properties of organisms relevant to bioprocessing

Property	*Relevance to bioprocessing*
Nature of strain, pathogenicity, toxin production, strain stability	Implications for safety and acceptability of strain in processing and in product quality assurance
Genetics	Product forming ability; media design related to induction and repression; potential for control, deregulation; genetic manipulation; genetic stability
Cell structure, shape, size, morphology	Implications for reactor design, cell separation, cell disintegration
Nature of cell membrane and transport systems	Capacity for substrate assimilation – with implications for media design and growth rate. Capacity for product secretion with implications for feedback regulation of synthesis and downstream processing
Cell nutrition	Catabolic and biosynthetic capabilities, media design
Gas requirements	Reactor design, types of metabolite produced, growth rate and yield
Cell growth kinetics	Fermentation time, substrate concentration, medium pH, culture temperature, potential for contamination by faster growing organisms
Relationship between growth and product formation	Fermentation process design
Presence of regulatory enzymes	Design and control of medium composition, substrate concentrations and other environmental conditions. Potential for deregulation
Activity of intracellular and extracellular proteases	Implications for protein turnover, enzyme half-lives, bioconversion rates, peptide product degradation

Cells may be distinguished on the basis of their gas requirements. Micro-organisms such as *Streptomyces* and most filamentous fungi are strict aerobes, i.e., they grow only in the presence of atmospheric oxygen. Clostridia, on the other hand, are strict anaerobes, growing only in the absence of oxygen. Facultative organisms, including industrial yeasts, can grow aerobically or anaerobically. Animal and plant cells also require oxygen to grow in culture. However, because culture growth rates are lower and, in the case of animal cells, densities are also low compared to micro-organisms, lower rates of oxygen transfer in bioreactors are required. Animal and plant cells are often gassed with carbon dioxide enriched air. Plant cell cultures may utilize some CO_2, while modulation of CO_2 gas flow may be used in animal cell culture to control pH. The gas requirements of cells in culture