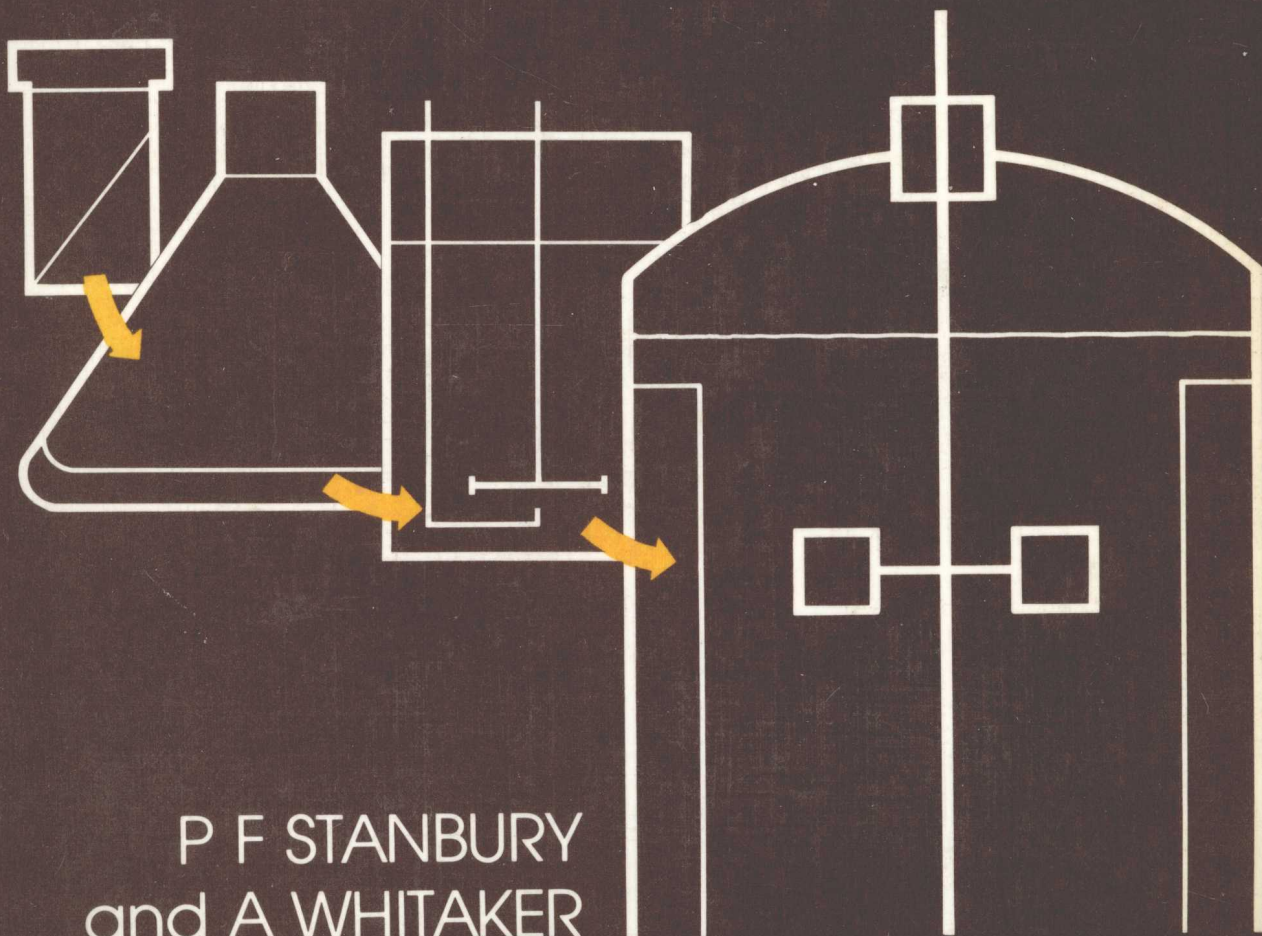


PERGAMON PRESS

Principles of Fermentation Technology



068505

Principles of Fermentation Technology

PETER F. STANBURY

B.Sc., M.Sc., D.I.C.

and

ALLAN WHITAKER

M.Sc., Ph.D., A.R.C.S., D.I.C.

Division of Biological and Environmental Sciences

Hatfield Polytechnic, Hertfordshire, UK



PERGAMON PRESS

OXFORD · NEW YORK · TORONTO · SYDNEY · PARIS · FRANKFURT

U.K.	Pergamon Press Ltd., Headington Hill Hall, Oxford OX3 0BW, England
U.S.A	Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, New York 10523, U.S.A.
CANADA	Pergamon Press Canada Ltd., Suite 104, 150 Consumers Road, Willowdale, Ontario M2J 1P9, Canada
AUSTRALIA	Pergamon Press (Aust.) Pty. Ltd., P.O. Box 544, Potts Point, N.S.W. 2011, Australia
FRANCE	Pergamon Press SARL, 24 rue des Ecoles, 75240 Paris, Cedex 05, France
FEDERAL REPUBLIC OF GERMANY	Pergamon Press GmbH, Hammerweg 6, D-6242 Kronberg-Taunus, Federal Republic of Germany

Copyright © 1984 Pergamon Press Ltd.

All Rights Reserved. No part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or by any means: electronic, electrostatic, magnetic tape, mechanical, photocopying, recording or otherwise, without permission in writing from the copyright holders.

First edition 1984

Library of Congress Cataloging in Publication Data

Stanbury, Peter F.

Principles of fermentation technology.

(Pergamon international library of science,
technology, engineering, and social studies)

Includes index.

I. Industrial microbiology.

I. Whitaker, Allan.

II. Title. III. Series.

QR53.S73 1984 660'.62 83-25665

British Library Cataloguing in Publication Data

Stanbury, Peter F.

Principles of fermentation technology.

—(Pergamon international library)

I. Industrial microbiology

I. Title II. Whitaker, Allan

660'.62 QR53

ISBN 0-08-024400-9

ISBN 0-08-024406-8 Pbk

**PERGAMON INTERNATIONAL LIBRARY
of Science, Technology, Engineering and Social Studies**

*The 1000-volume original paperback library in aid of education,
industrial training and the enjoyment of leisure*

Publisher: Robert Maxwell, M.C.

Principles of Fermentation Technology



THE PERGAMON TEXTBOOK INSPECTION COPY SERVICE

An inspection copy of any book published in the Pergamon International Library will gladly be sent to academic staff without obligation for their consideration for course adoption or recommendation. Copies may be retained for a period of 60 days from receipt and returned if not suitable. When a particular title is adopted or recommended for adoption for class use and the recommendation results in a sale of 12 or more copies, the inspection copy may be retained with our compliments. The Publishers will be pleased to receive suggestions for revised editions and new titles to be published in this important International Library.

Other Pergamon publications of related interest

Books

FOOD SCIENCE, 2nd Edition

Birch, G. C., Cameron, A. G. & Spencer, M.

CHEMICAL ENGINEERING

Coulson, J. M. & Richardson, J. F.

UNIT OPERATIONS IN FOOD PROCESSING, 2nd Edition

Earle, R. L.

THE SCIENCE OF FOOD, 2nd Edition

Gaman, P. M. & Sherrington, K. B.

FERMENTATION PRODUCTS

(Advances in Biotechnology, Volumes 1-3)

Moo-Young, M.

Journals (*free specimen copies gladly sent on request*)

BIOTECHNOLOGY ADVANCES

JOURNAL OF STORED PRODUCTS RESEARCH

Acknowledgements

We wish to thank the authors, publishers and manufacturing companies listed below for allowing us to reproduce either original or copyright material.

Authors

S. Aiba (Figs. 3.13, 7.8 and 8.3), S. I. Alikhanian (Table 3.4), F. H. Deindoerfer and E. L. Gaden (Fig. 9.14), F. H. Deindoerfer and A. E. Humphrey (Fig. 5.1), A. L. Demain (Fig. 3.12), E. L. Dulaney (Figs. 3.3 and 3.4), T. Jackson (Fig. 6.3), B. Liptak (Figs. 8.9, 8.10, 8.11, 8.12, 8.16 and 8.17), E. Mayer (Fig. 7.30), M. O. Moss (Tables 3.4 and 5.3), L. J. Nisbet (Tables 3.2 and 3.3), J. W. Richards (Figs. 5.2a, 5.2b, 5.2c, 5.3a, 5.3b, 5.7 and 7.13, and Table 5.2 from *Introduction to Industrial Sterilization*, Academic Press, London, 1968), S. R. L. Smith (Fig. 7.33b), D. I. C. Wang (Fig. 10.5), D. I. C. Wang and R. C. J. Fewkes (Fig. 9.15), A. Wiseman (Figs. 9.13 and 10.4).

Publishers and manufacturing companies

Academic Press, London: Fig. 1.2 from Turner, W. B. (1971) *Fungal Metabolites*; Fig. 6.1 from Rose, A. H. and Harrison, J. S. (1970) *The Yeasts*, Vol. 3; Figs. 6.2 and 6.5 from Norris, J. R. and Ribbons, D. W. (1972) *Methods in Microbiology*, Vol. 7B; Fig. 7.5 from Solomons, G. L. (1969) *Materials and Methods in Fermentation*; Fig. 7.29 from Rose, A. H. (1978) *Economic Microbiology*, Vol. 2; Fig. 7.34 from Rose, A. H. (1979) *Economic Microbiology*, Vol. 4; Fig. 12.1 from Nisbet, L. J. and Winstanley, D. J. (1984) *Bioactive Microbial Products*, Vol. 2; Table 12.2 from Rose, A. H. (1979) *Economic Microbiology*, Vol. 3.

Academic Press, New York: Fig. 10.5 from *Advances in Applied Microbiology* (1970), Vol. 12; Table 3.4 from *Advances in Applied Microbiology* (1962), Vol. 4.

American Society for Microbiology: Figs. 5.11, 9.14 and 9.15.

American Chemical Society: Table 6.2 from *Industrial and Engineering Chemistry* (1951), **43**, 1488–1498; Table 6.3 from *Industrial and Engineering Chemistry* (1952) **44**, 1677–1682; Fig. 7.8 from *Industrial and Engineering Chemistry* (1956) **48**, 2180–2182; Fig. 7.27 from *Industrial and Engineering Chemistry* (1951) **43**, 1702–1711.

Avi Publishing Co., PO Box 831, Westport, CT 066881, U.S.A.: Table 1.1 from Prescott and Dunn's *Industrial Microbiology*, edited by Reed, G. (1982).

Blackie and Son Ltd.: Fig. 10.7 reproduced with permission from Purchas, D. B. *Industrial Filtration of Liquids*, Leonard Hill, Glasgow and London, 1971.

Blackwell Scientific Publications: Figs. 1.1 and 7.9.

British Valves Manufacturers' Association Ltd.: Figs. 7.17, 7.18, 7.20, 7.22, 7.23 and 7.26.

Cambridge University Press: Fig. 3.29 from Society for General Microbiology Symposium, **29** *Microbial Technology: Current State, Future Prospects*.

Canadian Society of Chemical Engineering: Figs. 10.30a and 10.30b.

Chapman and Hall: Fig. 7.32.

Chilton Book Company Ltd., Radnor, Pennsylvania, U.S.A.: Figs. 8.9, 8.10, 8.11, 8.12, 8.16 and 8.17 reprinted from *Engineers Handbook*, Vol. 1, by B. Liptak, copyright 1969 by the author. Reprinted with the permission of the publisher.

C.R.C. Press, Inc., Boca Raton, Florida, U.S.A.: Fig. 2.10 reprinted with permission from Calcott, P.A. (1981) *Continuous Culture of Cells*, Vol. 1.

Elsevier Science Publishers, BV: Fig. 10.27.

Federation of European Biochemical Societies: *European Journal of Biochemistry*, Fig. 8.15.

Ellis Horwood: Figs. 9.13 and 10.4.

Institute of Water Pollution Control: Fig. 11.1.

Istituto Superiore di Sanita, Rome: Fig. 7.7.

Kodansha Scientific Ltd.: Fig. 3.13.

L. H. Engineering Ltd.: Figs. 7.3, 7.4 and 7.11.

McGraw Hill, New York: Fig. 7.25 reproduced with permission from King, R. C. (1967) *Piping Handbook*, 5th Edition, and also Fig. 10.9 from Perry, R. H. and Chilton, C. H. (1973) *Chemical Engineers Handbook*, 5th Edition.

Mechanical Engineering Publications Ltd., London: Figs. 7.19 and 7.21.

New York Academy of Sciences: Figs. 3.3 and 3.4

Pennwalt Ltd.: Figs. 10.15a, 10.15b, 10.17a and 10.17b.

Pergamon Press, Oxford: Figs. 7.24, 7.33a, 9.2, 10.8a, 10.8b and 10.31.

Royal Society of Chemistry, London: Figs. 6.4, 6.6 and 7.15. Table 6.5

The Royal Society, London: Fig. 7.33b.

Society for Industrial Microbiology: Fig. 9.15.

Tokyo University Press: Figs. 5.10a, 5.10b, 7.8 and 8.3.

Traders Protein Division: Table 4.8.

Wheatlands Journals Ltd.: Figs. 2.9, 5.4, 5.5 and 5.12.

John Wiley and Sons Inc., New York: Figs. 7.10, 7.28, 7.31, 8.19, 10.10, 10.11, 10.12, 10.18, 10.19 and 10.20. Tables 4.3, 5.1, 12.4 and 12.5.

We also wish to thank Dr T. Whitaker for advice on load cells and Fig. 8.14 a to h, and W. H. Mayes and Son, Windsor for photographs of load cells used for drawing Figs. 8.14g and 8.14h.

Last but not least we wish to express our thanks to Lesley, John, David and Abigail Stanbury and Lorna, Michael and Ben Whitaker for their encouragement and patience during all stages in the preparation of this book.

May, 1984

Contents

1	AN INTRODUCTION TO FERMENTATION PROCESSES	1
	The range of fermentation processes	1
	Microbial biomass	1
	Microbial enzymes	2
	Microbial metabolites	3
	Transformation processes	5
	The chronological development of the fermentation industry	5
	The component parts of a fermentation process	8
	References	9
2	MICROBIAL GROWTH KINETICS	11
	Batch culture	11
	Continuous culture	14
	Multistage systems	16
	Feedback systems	16
	The application of continuous culture in industrial processes	17
	Productivity	17
	Uniformity of operation and ease of automation	18
	Susceptibility of continuous fermentations to contamination	18
	Continuous culture and brewing	19
	Continuous culture and biomass production	20
	Application of continuous culture in strain isolation and improvement	21
	Application of continuous culture in the generation of basic information on commercial organisms	21
	Fed-batch culture	21
	Application of fed-batch culture	22
	Use of fed-batch culture in removal of repression and maintenance of aerobic conditions	22
	Use of fed-batch culture in the avoidance of toxic effects of medium components	24
	References	24
3	THE ISOLATION, PRESERVATION AND IMPROVEMENT OF INDUSTRIAL MICRO-ORGANISMS	26
	The isolation of industrially important micro-organisms	26
	isolation methods utilizing selection of the desired characteristic	27
	Enrichment liquid culture	27
	The use of solidified media	29
	Isolation methods not utilizing selection of the desired characteristic	30
	Screening for the production of antibiotics	30
	Screening for pharmacologically active compounds	30
	Screening for the production of growth factors	31
	Screening for the production of polysaccharides	31

Contents

The preservation of industrially important micro-organisms	31
Storage at reduced temperature	32
Storage on agar slopes	32
Storage of spores in water	32
Storage under liquid nitrogen	32
Storage in a dehydrated form	32
Soil culture	32
Lyophilization	32
Quality control of preserved stock cultures	32
The improvement of industrial micro-organisms	33
The selection of induced mutants synthesizing improved levels of primary metabolites	35
Modification of the permeability	37
The isolation of mutants which do not produce feedback inhibitors or repressors	38
Examples of the use of auxotrophs for the production of primary metabolites	40
The isolation of mutants that do not recognize the presence of inhibitors and repressors	43
The selection of induced mutants synthesizing improved levels of enzymes of industrial significance	47
Biosynthetic enzymes of primary metabolism	47
Catabolic enzymes	47
The isolation of induced mutants producing improved yields of secondary metabolites where directed selection is difficult to apply	49
The isolation of auxotrophic mutants	53
The isolation of resistant mutants	55
Mutants resistant to the analogues of primary metabolic precursors of the secondary metabolite	55
Mutants resistant to the feedback effects of the secondary metabolite	55
The isolation of mutants resistant to the toxic effects of the secondary metabolite in the trophophase	56
The isolation of mutants resistant to the presence of toxic precursors in the growth phase	56
The isolation of revertant mutants	57
The isolation of revertants of mutants auxotrophic for primary metabolites which may influence the production of a secondary metabolite	57
The isolation of revertants of mutants which have lost the ability to produce a secondary metabolite	57
The use of recombination systems for the improvement of industrial micro-organisms	58
The application of the parasexual cycle	58
The application of recombination systems in the actinomycetes	61
The application of protoplast fusion techniques	62
The application of genetic manipulation techniques	63
The improvement of industrial strains by modifying properties other than the yield of product	66
The selection of stable strains	66
The selection of strains resistant to infection	67
The selection of non-foaming strains	67
The selection of strains which are resistant to components in the medium	68
The selection of morphologically favourable strains	68
The selection of strains which are tolerant of low oxygen tension	69
The elimination of undesirable products from a production strain	69
The isolation of mutants producing new fermentation products	69
References	70

4	MEDIA FOR INDUSTRIAL FERMENTATIONS	74
	Introduction	74
	Typical media	74
	Medium formulation	75
	Water	77
	Energy sources	77
	Carbon sources	77
	Examples of commonly used carbon sources	77
	Factors influencing the choice of carbon source	79
	The influence of the carbon source on product formation	79
	Nitrogen sources	79
	Examples of commonly used nitrogen sources	79
	Factors influencing the choice of nitrogen source	80
	Minerals	81
	Vitamin sources	82
	Nutrient recycle	82
	Buffers	82
	The addition of precursors and metabolic regulators to media	83
	Precursors	83
	Inhibitors	83
	Inducers	84
	Oxygen requirements	85
	Fast metabolism	85
	Rheology	85
	Restricted nutrient levels	86
	Antifoams	86
	References	87
5	STERILIZATION	91
	Introduction	91
	Medium sterilization	92
	The design of batch sterilization processes	96
	Calculation of the Del factor during heating and cooling	98
	Calculation of the holding time at constant temperature (121°C)	98
	Richards' rapid method for the design of sterilization cycles	99
	The scale up of batch sterilization processes	99
	Methods of batch sterilization	100
	The design of continuous sterilization processes	100
	Sterilization of the fermenter	103
	Sterilization of the feeds	104
	Sterilization of air	104
	The theory of fibrous filters	104
	Filter design	106
	References	107
6	THE DEVELOPMENT OF INOCULA FOR INDUSTRIAL FERMENTATIONS	108
	Introduction	108
	The development of inocula for yeast processes	110
	Brewing	110
	Bakers' yeast	111

Contents

The development of inocula for bacterial processes	111
The development of inoculum for fungal processes	112
Sporulation on solidified media	112
Sporulation on solid media	113
Sporulation in submerged culture	113
The use of the spore inoculum	113
Inoculum development for vegetative fungi	114
The effect of the inoculum on the morphology of fungi in submerged culture	114
The development of inoculum for streptomycete processes	115
The aseptic inoculation of plant fermenters	116
Inoculation from a laboratory fermenter or a spore suspension vessel	116
Inoculation from a plant fermenter	118
References	118
 7 DESIGN OF A FERMENTER	 120
Introduction	120
Basic functions of a fermenter	121
Body construction	121
Aeration and agitation	124
The impeller (agitator)	124
Stirrer glands and bearings	125
The packed-gland seal (stuffing box)	126
Bush seals	126
The mechanical seal	127
Magnetic drives	127
Baffles	128
The aeration system (sparger)	128
Porous sparger	128
Orifice sparger	128
Nozzle sparger	129
Combined sparger–agitator	129
The achievement and maintenance of aseptic conditions	129
Sterilization of the fermenter	129
Sterilization of the air supply	129
Aeration and agitation	131
The addition of inoculum, nutrients and other supplements	131
Sampling	131
Foam control	132
Monitoring and control of various parameters	132
Valves	132
Gate valves	132
Globe valves	133
Piston valves	133
Plug valves	133
Needle valves	134
Butterfly valves	134
Ball valves	134
Pinch valves	135
Diaphragm valves	135

Check valves	135
Pressure-control valves	136
Pressure-reduction valves	136
Pressure-retaining valves	136
Safety valves	136
Other fermentation vessels	136
The packed tower	136
The tower fermenter	136
The Waldhof-type fermenter	137
Acetators and cavitators	137
The cyclone column	139
Cylindro-conical vessels	139
Air-lift fermenters	140
Deep-jet fermenter	141
Rotating-disc fermenters	141
References	142
 8 INSTRUMENTATION AND CONTROL	 145
Introduction	145
Control systems	145
Manual control	145
Automatic control	146
Two-position controllers (On/Off)	146
Proportional control	147
Integral control	148
Derivative control	149
Combinations of methods of control	149
Proportional plus integral control	149
Proportional plus derivative control	150
Proportional plus integral plus derivative control	150
Methods of measurement for process variables	150
Temperature	150
Mercury-in-glass thermometers	150
Bimetallic thermometers	150
Pressure bulb thermometers	150
Thermocouples	150
Electrical resistance thermometers	151
Thermistors	151
Temperature control	151
Flow measurement and control	151
Gases	151
Liquids	152
Pressure measurement	153
Pressure control	154
Safety valves	155
Agitator-shaft power	155
Rate of stirring	155
Foam sensing and control	155
Weight	156

Contents

Measurement and control of dissolved oxygen	158
Exit-gas analysis	159
pH measurement and control	161
Redox	161
Carbon dioxide electrodes	162
On-line analysis of other chemical factors	162
Ion-specific sensors	162
Enzyme electrodes	162
Microbial electrodes	162
Mass spectrometers	163
Fluorimeters	163
Computer applications in fermentation technology	163
Components of a computer-linked system	164
Data logging	165
Data analysis	165
Process control	166
References	167
 9 AERATION AND AGITATION	 169
Introduction	169
The oxygen requirements of industrial fermentations	169
Oxygen supply	172
Determination of K_La values	173
The sulphite oxidation technique	173
Gassing-out techniques	174
The static method of gassing out	174
The dynamic method of gassing out	175
The oxygen-balance technique	177
Fluid rheology	178
Bingham plastic rheology	179
Pseudoplastic rheology	180
Dilatant rheology	180
Casson body rheology	180
Factors affecting K_La values in fermentation vessels	181
The effect of air-flow rate on K_La	181
The effect of the degree of agitation on K_La	181
The relationship between K_La and power consumption	182
The relationship between power consumption and operating variables	183
The effect of medium and culture rheology on K_La	186
Medium rheology	186
The effect of microbial biomass on K_La	187
The effect of microbial products on aeration efficiency	190
The effect of foam and antifoams on oxygen transfer	190
References	191
 10 THE RECOVERY AND PURIFICATION OF FERMENTATION PRODUCTS	 193
Introduction	193
Removal of microbial cells and other solid matter	195

Foam separation	195
Precipitation	196
Filtration	196
Theory of filtration	196
The use of filter aids	198
Batch filters	198
Plate frame filters	198
Pressure leaf filters	199
Vertical metal-leaf filters	199
Horizontal metal-leaf filters	199
Stacked-disc filters	199
Continuous filters	200
Rotary vacuum filters	200
String discharge	200
Scraper discharge	200
Scraper discharge with precoating of the drum	200
The centrifuge	201
Cell aggregation and flocculation	201
The range of centrifuges	202
The basket centrifuge (perforated-bowl basket centrifuge)	202
The multichamber centrifuge	202
Solid-bowl scroll centrifuge (decanter centrifuge)	203
Disc-bowl centrifuge	204
The tubular-bowl centrifuge	204
Cell disruption	206
Physical-mechanical methods	206
Liquid shear	206
Solid shear	206
Agitation with abrasives	207
Freezing-thawing	207
Chemical methods	207
Detergents	207
Osmotic shock	207
Alkali treatment	208
Enzyme treatment	208
Liquid-liquid extraction	208
Solvent recovery	211
Chromatography	212
Adsorption chromatography	213
Ion exchange	213
Gel filtration	214
Affinity chromatography	214
Continuous chromatography	214
Ultra filtration	215
Drying	215
Crystallization	216
Whole broth processing	216
References	217

11	EFFLUENT TREATMENT	220
	Introduction	220
	Dissolved oxygen concentration as an indicator of water quality	220
	Factory surveys	221
	The strengths of fermentation effluents	222
	Treatment and disposal of effluents	222
	Disposal	223
	Seas and rivers	223
	Lagoons	223
	Spray irrigation	224
	Well disposal	224
	Disposal of effluents to sewers	224
	Treatment processes	224
	Physical treatment	224
	Chemical treatment	225
	Biological treatment	225
	Aerobic processes	225
	Trickling filters	225
	Towers	226
	Rotating discs	226
	Rotating drums	227
	Activated sludge	227
	Anaerobic treatment	228
	Anaerobic digestion	228
	Anaerobic filters	228
	By-products	228
	Distilleries	229
	Breweries	229
	Amino acid wastes	229
	References	229
12	FERMENTATION ECONOMICS	231
	Introduction	231
	Isolation of micro-organisms of potential industrial interest	232
	Strain improvement	233
	Market potential	233
	Plant and equipment	235
	Media	236
	Air sterilization	237
	Heating and cooling	238
	Aeration and agitation	238
	Batch-process cycle times	240
	Continuous culture	240
	Recovery costs	241
	Water usage and recycling	242
	Effluent treatment	242
	References	243
	Index	247

CHAPTER 1

An Introduction to Fermentation Processes

THE term 'fermentation' is derived from the Latin verb, *fervere*, to boil, thus describing the appearance of the action of yeast on extracts of fruit or malted grain. The boiling appearance is due to the production of carbon dioxide bubbles caused by the anaerobic catabolism of the sugars present in the extract. However, fermentation has come to have different meanings to biochemists and to industrial microbiologists. Its biochemical meaning relates to the generation of energy by the catabolism of organic compounds, whereas its meaning in industrial microbiology tends to be much broader.

The catabolism of sugars is an oxidative process which results in the production of reduced pyridine nucleotides which must be reoxidized for the process to continue. Under aerobic conditions, reoxidation of reduced pyridine nucleotide occurs by electron transfer, via the cytochrome system, with oxygen acting as the terminal electron acceptor. However, under anaerobic conditions, reduced pyridine nucleotide oxidation is coupled with the reduction of an organic compound, which is often a subsequent product of the catabolic pathway. In the case of the action of yeast on fruit or grain extracts, NADH is regenerated by the reduction of pyruvic acid to ethanol. Different microbial taxa are capable of reducing pyruvate to a wide range of end products, as illustrated in Fig. 1.1. Thus, the term fermentation has been used in a strict biochemical sense to mean an energy-generating process in which organic compounds act as both electron donors and terminal electron acceptors.

The production of alcohol by the action of yeast on malt or fruit extracts has been carried out on a

large scale for very many years and was the first 'industrial' process for the production of a microbial metabolite. Thus, industrial microbiologists have extended the term fermentation to describe any process for the production of product by the mass culture of a micro-organism. Brewing and the production of organic solvents may be described as fermentations in both senses of the word but the description of an aerobic process as a fermentation is obviously using the term in the broader, microbiological, context and it is in this sense that the term is used in this book.

THE RANGE OF FERMENTATION PROCESSES

There are four major groups of commercially important fermentations:

- (i) Those that produce microbial cells (or biomass) as the product.
- (ii) Those that produce microbial enzymes.
- (iii) Those that produce microbial metabolites.
- (iv) Those that modify a compound which is added to the fermentation—the transformation processes.

The historical development of these processes will be considered in a later section of this chapter, but it is first necessary to include a brief description of the four groups.

Microbial biomass

The commercial production of microbial biomass may be subdivided into two major processes; the

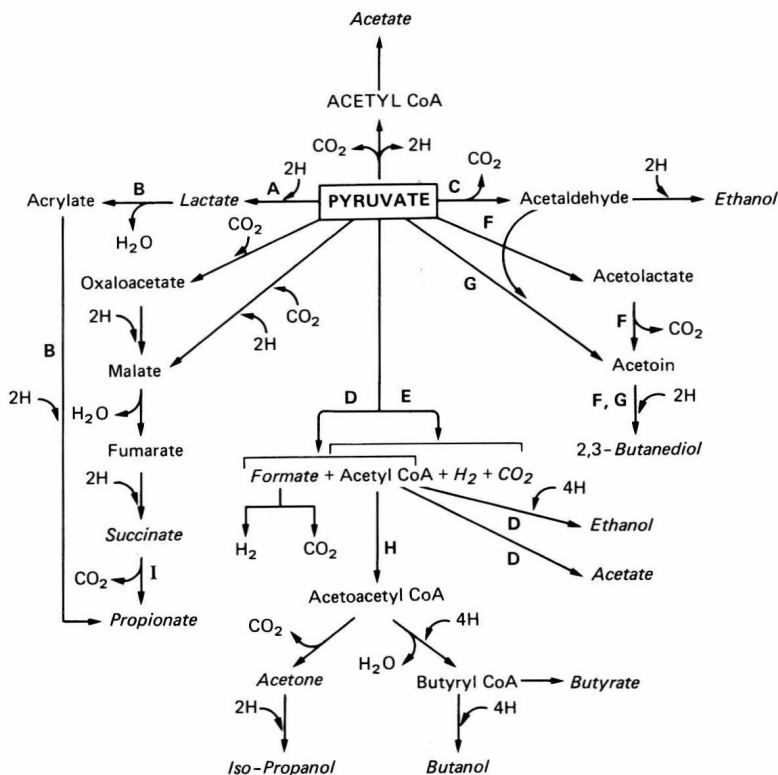


FIG. 1.1. Bacterial fermentation products of pyruvate. Pyruvate formed by the catabolism of glucose is further metabolized by pathways which are characteristic of particular organisms and which serve as a biochemical aid to identification. End products of fermentations are italicized (Dawes and Large, 1982).

- A Lactic acid bacteria (*Streptococcus*, *Lactobacillus*)
 B *Clostridium propionicum*
 C Yeast, *Acetobacter*, *Zymomonas*, *Sarcina ventriculi*, *Erwinia amylovora*
 D Enterobacteriaceae (coliforms)
 E Clostridia

- F *Klebsiella*
 G Yeast
 H Clostridia (butyric, butylic organisms)
 I Propionic acid bacteria

production of yeast to be used in the baking industry and the production of microbial cells to be used as human or animal food (single-cell protein). Bakers' yeast has been produced on a large scale since the early 1900s and yeast was produced as human food in Germany during the First World War. However, it was not until the 1960s that the production of microbial biomass as a source of food protein was explored to any great depth. As a result of this work, reviewed briefly in Chapter 2, a few very large-scale continuous processes have been established in recent years using a variety of different carbon sources.

Microbial enzymes

Enzymes have been produced commercially from plant, animal and microbial sources. However,

microbial enzymes have the enormous advantage of being able to be produced in large quantities by established fermentation techniques. Also, it is infinitely easier to improve the productivity of a microbial system compared with a plant or animal one. The uses to which microbial enzymes have been put are summarized in Table 1.1, from which it may be seen that the majority of applications are in the food and related industries. Enzyme production is closely controlled in micro-organisms and in order to improve productivity these controls may have to be exploited or modified. Such control systems as induction may be exploited by including inducers in the medium, whereas feedback repression may be removed by mutation and selection techniques. These aspects are discussed in Chapters 4 and 3, respectively.

TABLE 1.1. *Commercial applications of enzymes* (Boing, 1982)

Industry	Application	Enzyme	Source
Baking and milling	Reduction of dough viscosity, acceleration of fermentation process, increase in loaf volume, improvement of crumb score and softness, maintenance of freshness and softness	Amylase	Fungal
	Improvement of dough texture, reduction of mixing time, increase in loaf volume	Protease	Fungal/bacterial
Beer	Mashing	Amylase	Fungal/bacterial
	Chillproofing	Protease	Fungal/bacterial
	Improvement of fine filtration	β -Glucanase	Fungal/bacterial
Cereals	Precooked baby foods, breakfast foods	Amylase	Fungal
	Condiments	Protease	Fungal/bacterial
Chocolate, cocoa	Manufacture of syrups	Amylase	Fungal/bacterial
Coffee	Coffee bean fermentation	Pectinase	Fungal
	Preparation of coffee concentrates	Pectinase, hemicellulase	Fungal
Confectionery, candy	Manufacture of soft-centre candies and fondants	Invertase, pectinase	Fungal/bacterial
	Sugar recovery from scrap candy	Amylase	Fungal/bacterial
Corn syrup	Manufacture of high-maltose syrups	Amylase	Fungal
	Production of low D.E. syrups	Amylase	Bacterial
	Production of glucose from corn syrup	Amyloglucosidase	Fungal
	Converting corn syrup to a sweeter fructose-containing product	Glucose isomerase	Bacterial
Dairy	Residual H_2O_2 removal from milk (subsequent to sterilization by H_2O_2)	Catalase	Fungal
	Manufacture of protein hydrolysates	Protease	Fungal/bacterial
	Stabilization of evaporated milk	Protease	Fungal
	Production of whole milk concentrates, whey concentrates, and icecream and frozen desserts	Lactase	Yeast
	Curdling milk	Protease	Fungal/bacterial
Distilled beverages	Mashing	Amylase	Fungal/bacterial
Eggs, dried	Glucose removal	Glucose oxidase	Fungal
Feeds, animal	Pig starter rations	Amylase, protease	Fungal
Flavours	Clarification (starch removal)	Amylase	Fungal
	Oxygen removal	Glucose oxidase	Fungal
Fruit juices	Clarification, preventing gelling of concentrates, improvement of juice extraction yield	Pectinases	Fungal
	Oxygen removal	Glucose oxidase	Fungal
Laundry	Detergents	Protease	Bacterial
Leather	Dehairing, bating	Protease	Fungal/bacterial
Meat	Tenderization	Protease	Fungal
	Preparation of fish protein concentrates	Protease	Fungal/bacterial
Pharmaceutical and clinical	Digestive aids	Amylase, protease	Fungal
	Injection for bruises, inflammation, etc.	Streptokinase	Bacterial
	Various clinical tests	Numerous	Fungal/bacterial
Photography	Recovery of silver from spent film	Protease	Bacterial
Protein hydrolysates	Preparation of protein hydrolysates	Protease	Fungal/bacterial
Soft drinks	Stabilization of citrus terpenes from light-catalysed oxidation	Glucose oxidase and catalase	Fungal
Textiles	Desizing of fabrics	Amylase	Bacterial
Vegetables	Preparation of hydrolysates	Pectinase, cellulase	Fungal
	Liquefying purees and soups	Amylase	Fungal
Wine	Clarification of must	Pectinase	Fungal

Microbial metabolites

The growth of a microbial culture may be divided into a number of stages, as discussed in Chapter 2. After the inoculation of a culture into a nutrient

medium there is a period during which growth does not appear to occur; this period is referred to as the lag phase and may be considered as a time of adaptation. Following a period during which the growth rate of the cells gradually increases the cells