

Biochemistry of Industrial Micro-organisms

Edited by C. RAINBOW and A. H. ROSE

Biochemistry of Industrial Micro-organisms

Edited by

C. RAINBOW

Bass, Ratcliff and Gretton Limited, Burton-on-Trent, England

and

A. H. ROSE

*Department of Microbiology, University of
Newcastle-upon-Tyne, England*



ACADEMIC PRESS

LONDON and NEW YORK · 1963

ACADEMIC PRESS INC. (LONDON) LTD.
BERKELEY SQUARE HOUSE
LONDON, W.1

U.S. Edition published by
ACADEMIC PRESS INC.
111 FIFTH AVENUE
NEW YORK 3, NEW YORK

Copyright © 1963 by Academic Press Inc. (London) Ltd.

All rights reserved

NO PART OF THIS BOOK MAY BE REPRODUCED IN ANY FORM, BY
PHOTOSTAT, MICROFILM, OR ANY OTHER MEANS, WITHOUT
WRITTEN PERMISSION FROM THE PUBLISHER

Library of Congress Catalog Card Number: 63-21393

Printed in Great Britain by Spottiswoode, Ballantyne & Co. Ltd.,
London and Colchester

Contributors

- R. F. ANDERSON, *Northern Utilization Research and Development Division, United States Department of Agriculture, Agricultural Research Service, Peoria, Illinois, U.S.A.* (p. 300)
- J. H. BIRKINSHAW, *Department of Biochemistry, London School of Hygiene and Tropical Medicine, University of London, London, England* (p. 452)
- H. J. BUNKEB, *Consultant in Microbiology, Twickenham, Middlesex, England* (p. 34)
- R. DAVIES, *Medical Research Council Unit for Chemical Microbiology, Department of Biochemistry, University of Cambridge, Cambridge, England* (p. 68)
- B. P. EDDY (the late), *Low Temperature Research Station, University of Cambridge, and Agricultural Research Council, Cambridge, England* (p. 489)
- T. W. GOODWIN, *Department of Agricultural Biochemistry, Institute of Rural Science, University College of Wales, Aberystwyth, Wales* (p. 151)
- J. F. GROVE, *Akers Research Laboratories, Imperial Chemical Industries Ltd., Pharmaceutical Division, Welwyn, Herts., England* (p. 320)
- A. N. HALL, *Department of Biochemistry, Manchester College of Science and Technology, Manchester, England* (p. 607)
- J. S. HARRISON, *Research and Development Department, The Distillers Company Ltd., Epsom, Surrey, England* (p. 9)
- D. J. D. HOCKENHULL, *Glaxo Laboratories Ltd., Ulverston, Lancashire, England* (p. 227)
- D. JENKINS, *Sanitary Engineering Research Laboratory, University of California, Berkeley, California, U.S.A.* (p. 508)
- S. KINOSHITA, *Tokyo Research Laboratory, Kyowa Hakko Kogyo Co. Ltd., Tokyo, Japan* (p. 206)
- S. M. MARTIN, *Division of Applied Biology, National Research Council of Canada, Ottawa, Canada* (p. 415)
- D. H. PETERSON, *The Upjohn Company, Kalamazoo, Michigan, U.S.A.* (p. 537)
- C. RAINBOW, *The Laboratory, Bass, Ratcliff and Gretton, Burton-on-Trent, Staffordshire, England* (p. 1)
- A. H. ROSE, *Department of Microbiology, University of Newcastle-upon-Tyne, England* (p. 1 and p. 379)
- W. A. TABER, *Prairie Regional Laboratory, National Research Council of Canada, Saskatoon, Saskatchewan, Canada* (p. 341)
- L. C. VINING, *Atlantic Research Laboratory, National Research Council of Canada, Halifax, Nova Scotia, Canada* (p. 341)
- J. F. WILKINSON, *Department of Bacteriology, University of Edinburgh, Edinburgh, Scotland* (p. 379)

Preface

There are probably few better documented examples of Man's scientific and technological development than the harnessing of the activities of micro-organisms. Although micro-organisms were first applied over 6000 years ago to brew beer and to make wine, it has been only during the present century that the potential value of this exploitation of microbial activity has begun to be realized. Developments over the past few decades have led to processes for making a wide variety of industrial chemicals, including organic acids, vitamins, amino acids and enzymes. Probably the most significant development was the discovery of antibiotics, a discovery which established a close liaison between the microbiological and pharmaceutical industries. This liaison has since led to further notable advances in the industrial applications of micro-organisms, particularly in the field of steroid transformations. There has been also a change in our concept of the "industrial micro-organism". Some 20-30 years ago, there existed a small band of micro-organisms—some yeasts and a few bacteria and moulds—that were conveniently referred to as industrial micro-organisms. But today this term can no longer be justifiably reserved for this elite. Indeed, it would now seem that every micro-organism is potentially of industrial importance; once it has been shown to produce a commercially important chemical, or to bring about a chemical transformation that can be applied in the synthesis of an industrially important substance, then the potential of that micro-organism can be realized.

Although the size and scope of the microbiological industries have undergone a tremendous metamorphosis in recent years, the rationale behind the use of micro-organisms in industrial processes remains the same, namely, the exploitation of their chemical activities. Several recent developments have been possible only because we have had a basic understanding of the chemical activities of the micro-organisms involved. And there is every reason to believe that still greater rewards can be expected if research and development are based on a firm knowledge of microbial biochemistry. It is for this reason that the industrial applications of micro-organisms are here described in terms of their biochemical activities.

In order that this book should be of the greatest value to those concerned with industrial micro-organisms, it was essential that it be written not by one or two authors but by a team of contributors, each a specialist in his own field. Throughout its preparation, it has been our aim to allow contributors a wide degree of latitude in interpreting developments in their subject and in the manner in which these developments are presented. We should like to thank all contributors for their willing co-operation in this venture which, we hope, will be of value not only to industrial microbiologists, but also to the whole community of microbiologists.

June, 1963

C. RAINBOW
A. H. ROSE

List of Common Abbreviations and Symbols

AMP, CMP, GMP, IMP, UMP	5' Phosphates of ribosyl-adenine, -cytosine, -guanine, -hypoxanthine, and -uracil
ADP, CDP, GDP, IDP, UDP	Adenosine, cytosine, guanosine, inosine and uridine diphosphates
ATP, CTP, GTP, ITP, UTP	Adenosine, cytosine, guanosine, inosine and uridine triphosphates
ABS	Alkyl benzene sulphonates
6-APA	6-Aminopenicillanic acid
BOD	Biochemical Oxygen Demand
CDPG	Cytidine diphosphate glucose
CoA, CoASH	Coenzyme A
DAP	Diaminopimelic acid
DNA	Deoxyribonucleic acid
EDTA	Ethylenediamine tetra-acetic acid
EMP	Embden-Meyerhof-Parnas pathway
FAD, FADH ₂	Flavin adenine dinucleotide, oxidized and reduced forms
FMN	Flavin monophosphate
GSSG, GSH	Glutathione, oxidized and reduced forms
HMP	Hexose monophosphate shunt
MVA	Mevalonic acid
NAD, NADH ₂	Nicotinamide adenine dinucleotide, oxidized and reduced forms
NADP, NADPH ₂	Nicotinamide adenine dinucleotide phosphate, oxidized and reduced forms
~ P	Energy-rich phosphate bond
P _i	Inorganic phosphate
PP	Inorganic pyrophosphate
RNA	Ribonucleic acid
TCA	Tricarboxylic acid cycle
TPP	Thiamine pyrophosphate
UDPG	Uridine diphosphate glucose

Contents

Contributors	v
Preface	vii

1. INTRODUCTION

A. H. Rose and C. Rainbow

2. BAKER'S YEAST

J. S. Harrison

I. Introduction	9
II. Basic Principles.	10
A. Chemical Anatomy of the Yeast Cell	10
B. Yeast Enzymes	11
C. Uptake of Nutrients and Excretion of Metabolic Products	11
D. Carbon Utilization	14
III. Synthesis of Cell Material	16
A. Carbohydrates	16
B. Nitrogen-containing Cell Constituents	17
IV. Growth	20
A. Vegetative Propagation.	20
B. Oxygen Requirements	22
C. Growth Factors	23
V. Reproduction	23
A. Genetics	23
B. Cell Division	24
C. Hybridization	25
VI. Fermentation	26
VII. Conclusions	29
References	30

3. MICROBIAL FOOD

H. J. Bunker

I. Introduction	34
II. The Case for Microbial Food	34

III.	Production of Essential Nutrients	35
	A. Proteins	35
	B. Lipids	47
	C. Vitamins	53
	D. Carbohydrates	61
IV.	Acceptability of Microbial Food	61
	References	63

4. MICROBIAL EXTRACELLULAR ENZYMES, THEIR USES AND SOME FACTORS AFFECTING THEIR FORMATION

R. Davies

I.	Introduction and General Considerations	68
II.	Uses of Microbial Extracellular Enzymes	70
III.	Production of Microbial Extracellular Enzymes	72
	A. Survey of Previous Literature	72
	B. Factors Affecting Synthesis of Extracellular Enzymes	74
IV.	Extracellular Enzymes of Bacteria and Fungi	104
	References	135

5. VITAMINS

T. W. Goodwin

I.	Introduction	151
II.	Riboflavin (Vitamin B ₂)	151
	A. Introduction	151
	B. Flavinogenic Organisms	152
	C. Biosynthesis	158
III.	Cobamides (Vitamins B ₁₂)	169
	A. Introduction	169
	B. Chemistry and Nomenclature	171
	C. Biosynthesis	175
IV.	Carotenoids	182
	A. Introduction	182
	B. General Factors Controlling Microbial Production of β -carotene	185
	C. Biosynthesis	187
	References	198

6. AMINO ACIDS

Shukuo Kinoshita

I.	Introduction	206
II.	L-Glutamate	207

III.	L-Lysine	216
IV.	L-Valine	218
V.	L-Ornithine	218
VI.	L-Homoserine	219
VII.	L-Threonine	219
VIII.	L-Isoleucine	220
IX.	Miscellaneous Amino Acids	220
	A. Alanine	220
	B. L-Tryptophan	221
	C. L-Phenylalanine	221
	D. L-Tyrosine	221
	E. L-Aspartate	221
X.	Future Prospects for the Microbiological Production of Amino Acids	221
	References	223

7. ANTIBIOTICS

D. J. D. Hockenhull

I.	Introduction	227
II.	Penicillin	228
	A. Production	228
	B. Biosynthesis	239
III.	Griseofulvin	246
	A. Production	246
	B. Biosynthesis	247
IV.	Streptomycin	249
	A. Production	249
	B. Biosynthesis	255
	C. Mannosidostreptomycin	266
	D. Other Streptomycins	267
V.	Tetracyclines	267
	A. Production	268
	B. Biosynthesis	272
VI.	Macrolides	276
	A. Production	278
	B. Biosynthesis	281
VII.	Chloramphenicol	284
	A. Production	285
	B. Biosynthesis	286
VIII.	Novobiocin	287
	A. Production	288
	B. Biosynthesis	289
IX.	Conclusion	290
	References	291

8. POLYSACCHARIDES

Ralph F. Anderson

I. Introduction	300
II. Historical Background	302
III. Biosynthesis of Homopolysaccharides from Disaccharides	302
A. Dextranucrase	303
B. Levansucrase	305
C. Amylomaltase	307
D. Amylosucrase	308
IV. Biosynthesis of Polysaccharides from Monosaccharides	309
A. Cellulose	309
B. Heteropolysaccharides	312
C. Developmental Research on Microbial Gums	314
References	316

9. GIBBERELLINS

John Frederick Grove

I. Introduction	320
II. Fungal Gibberellins	321
A. Historical Background	321
B. Physical and Chemical Properties	323
C. Detection and Estimation	324
D. Other Metabolic Products of <i>Gibberella fujikuroi</i>	326
III. Gibberellin Production by <i>Gibberella fujikuroi</i>	327
A. Surface Culture	327
B. Shake Culture	328
C. Stirred Culture	328
IV. Biosynthesis of the Gibberellins	331
References	338

10. ALKALOIDS

L. C. Vining and W. A. Taber

I. Introduction	341
II. Ergoline Alkaloids	342
A. History	342
B. Chemical Structure	345
C. Detection and Assay	350
D. Sources	351

E. Nutrition of <i>Claviceps</i> sp.	353
F. Metabolic Pathways in <i>Claviceps</i> sp.	354
G. Saprophytic Production	357
H. Physiology of Alkaloid Production	362
I. Biosynthesis	365
III. Psilocybin and Psilocin	370
A. History and Sources	370
B. Production	371
C. Isolation, Structure and Properties	371
D. Biosynthesis	372
References	373

II. FERMENTATION PROCESSES

J. F. Wilkinson and A. H. Rose

I. Introduction	379
II. Biochemistry of Fermentations	380
A. Carbohydrate Fermentations	380
B. Fermentation of Nitrogenous Substances	410
References	412

12. PRODUCTION OF ORGANIC ACIDS BY MOULDS

S. M. Martin

I. Introduction	415
II. Pathways of Glucose Metabolism	417
A. Glycolysis	417
B. Pentose Phosphate Cycle	420
C. Entner-Doudoroff Pathway	424
D. Non-phosphorylative Glucose Oxidation	425
III. Products of Glucose Metabolism	426
A. Gluconic Acid	427
B. Kojic Acid	429
C. Lactic Acid	430
IV. Products of the Tricarboxylic Acid and Glyoxylate Cycles	432
A. Biochemistry of the Cycles	432
B. Industrially Important Products	438
V. Miscellaneous Products	446
A. Gallic Acid	447
B. Ustilagic Acid	447
C. Gibberellic Acid	448
References	448

13. MISCELLANEOUS PRODUCTS OF FUNGAL METABOLISM

J. H. Birkinshaw

I.	Introduction	453
II.	Aliphatic Metabolites	453
	A. Unsaturated Compounds	453
	B. Products Related to Citric Acid	455
III.	Heterocyclic Rings Containing One Oxygen Atom	457
	A. Epoxides	457
	B. Furane Rings	457
	C. Tetronic Acids	458
	D. Pyrones	459
IV.	Aromatic Metabolites	460
	A. Monocyclic Structures	460
	B. Biosynthesis of Phenolic Metabolites	463
	C. Bi- and Tri-cyclic Metabolites	467
V.	Quinones	469
	A. Benzoquinones	469
	B. Naphthaquinones	470
	C. Anthraquinones	470
VI.	Tropolones	473
VII.	Metabolites Containing Nitrogen	473
	A. Volatile Amines	473
	B. Oligopeptides	474
	C. Quaternary Ammonium Compounds	475
	D. Pyridine Derivatives	476
	E. Quinoline Derivatives	476
	F. Nitrogen Metabolites Related to Quinones	477
	G. Nitrogen Metabolites Related to Tetronic Acids	478
	H. Diazine Rings	478
	I. Indole Derivatives	479
	J. Unusual Types of Nitrogen Compounds	480
VIII.	Metabolites Containing Sulphur	481
IX.	Metabolites Containing Chlorine	482
X.	Metabolites Containing Arsenic etc.	483
XI.	Function	484
	References	484

14. MICROBIAL SPOILAGE

the late B. P. Eddy

I.	Introduction	489
II.	Carbohydrates and Related Substances	490
	A. Cellulose	491
	B. Wood—Hemicelluloses and Lignin	493
	C. Pectin	494

CONTENTS

xv

D. Starch	495
E. Monosaccharides and Disaccharides	495
III. Proteins	495
A. Proteolysis	496
B. Putrefaction	499
IV. Lipids and Allied Compounds	500
A. Fats and Oils	500
B. Lecithin	502
V. Miscellaneous Types of Spoilage	503
VI. Conclusion	504
References	506

15. SEWAGE TREATMENT

D. Jenkins

I. Introduction	508
II. Nature of Sewage	509
III. Sewage Treatment Processes	511
A. Primary Treatment	511
B. Settled Sewage Treatment	511
C. Sludge Treatment	514
IV. Biological Populations of Sewage Treatment Plants	515
A. Activated Sludge	515
B. Biological Filters	518
V. Oxygen and Nutrient Supply	521
A. Oxygen	521
B. Nutrients	521
VI. Microbiological Activities	522
A. Removal of Organic Matter	522
B. Inorganic Nitrogen Metabolism	524
C. Sludge Digestion and Methane Formation	528
D. Breakdown of Synthetic Detergents	531
VII. Conclusions	533
VIII. Acknowledgements	534
References	534

16. MICROBIAL TRANSFORMATIONS OF STEROIDS AND THEIR APPLICATION TO THE PREPARATION OF HORMONES AND DERIVATIVES

Durey H. Peterson

I. Introduction	538
II. Oxidation Reactions	545
A. Simple Hydroxylations	545
B. Polyhydroxylations	557

C.	Introduction of Oxygen as a Ketone	558
D.	Epoxidations	559
E.	Simple Side Chain Degradation with or without Lactonization of Ring D	560
F.	Side Chain Degradations with Hydroxylation	562
G.	Introduction of Nuclear Double Bonds with no Side Chain Degradation	562
H.	Introduction of Nuclear Double Bonds and Side Chain Degradation	563
I.	Introduction of Nuclear Double Bonds with Hydroxylation of 3-Oxo- Δ^4 Steroids	566
J.	Oxidation of Nuclear and Side Chain Hydroxyl Groups	567
K.	Opening of Rings, A, B and D	568
III.	Reduction Reactions	571
A.	Reduction of Side Chain Carbonyl Groups	571
B.	Reduction of Nuclear Double Bonds, Nuclear Ketones and Combinations thereof.	573
IV.	Mixed Reactions	574
A.	Reduction of Nuclear Double Bonds and Hydroxylation	574
B.	Reduction of Carbonyl Groups and Hydroxylation	575
C.	Reduction of Carbonyl Groups and Introduction of Nuclear Double Bonds	575
D.	Aromatization	575
E.	Reversible Oxidation-Reduction Reactions	576
V.	Microbial Transformation of Cardiac Aglycones and Steroid Alkaloids	576
A.	Digitoxigenin and Related Compounds	576
B.	Steroid Alkaloids	576
VI.	Esterification Reactions	577
VII.	Esterases, Glucuronidases and other Enzymes	578
VIII.	Steroid Decarboxylase	579
IX.	Preparation of Steroid Hormones and Derivatives	580
A.	Naturally Occurring Adrenal Corticosteroids	580
B.	Modified Adrenal Corticosteroids	583
C.	Gonadal Hormones and Derivatives	585
X.	Microbial Enzymes in Structure Proof and Classification of Micro-organisms	589
XI.	Enzyme Mechanisms	589
XII.	Acknowledgements	597
	References	597

17. MISCELLANEOUS OXIDATIVE TRANSFORMATIONS

A. N. Hall

I.	Introduction	607
II.	Ketogenic Transformations of Polyols	608
A.	Transformation of Sugar Alcohols in Growing Cultures	608
B.	Polyol Dehydrogenases of Micro-organisms	610

C.	Conversion of Sorbitol to L-Sorbose	612
D.	Conversion of Glycerol to Dihydroxyacetone	612
E.	Oxidation of Cyclitols by <i>Acetobacter suboxydans</i>	613
III.	Some Transformation Products of Reducing Sugars	614
A.	5-Oxo-D-gluconic Acid	614
B.	2-Oxo-D-gluconic Acid	615
C.	2,5-Dioxogluconic Acid	616
D.	2-Oxo-L-gulonic Acid	617
E.	L-Ascorbic Acid and D-araboascorbic Acid	618
F.	Keto-sugars	618
G.	2-Oxoglutaric acid	619
H.	L-1-Phenyl-1-acetylcarbinol	619
IV.	Conversion of 2-Oxoglutaric Acid to L-Glutamic Acid	621
V.	Oxidative Transformations of <i>n</i> -Alkanes	621
A.	Subterminal Oxidation	621
B.	Terminal Oxidation	621
C.	Di-terminal Oxidation	623
VI.	Transformation of Alkaloids	623
A.	Yohimbine	623
B.	Nicotine	624
	References	625
	Author Index	629
	Subject Index	669

CHAPTER 1

Introduction

A. H. ROSE and C. RAINBOW

*Department of Microbiology, University of Newcastle-upon-Tyne, England,
and The Laboratory, Bass, Ratcliff and Gretton, Burton-on-Trent, England*

The harnessing of the activities of micro-organisms represents one of the most fascinating aspects of Man's scientific and technological development. Included among the industrial applications of micro-organisms are some of the oldest of Man's social and domestic activities, but it is only during the past few decades, following upon the dramatic advances that have been made in the science of biochemistry, that we have begun to appreciate fully the advantages that can be gained from this exploitation of microbial activity. The variety of ways in which micro-organisms have been used industrially are described in detail in several textbooks, including those of Underkofler and Hickey (1954) Prescott and Dunn (1959), Whitmarsh (1958) and Rose (1961). Each industrial microbiological process exploits the biochemical properties of a certain micro-organism or occasionally, as in sewage purification, of a mixed flora of micro-organisms.

The reasons why micro-organisms have proved such valuable agents in industrial processes are threefold and are based on those properties which distinguish them from higher plants and animals. A review by Gunsalus and Shuster (1961) deals with these distinguishing features in greater detail than is possible in this chapter.

Speed of Microbial Metabolism

Micro-organisms are extremely small with, on a weight-for-weight basis, a very high surface:volume ratio as compared with higher organisms. Because of the tremendous area available for absorption of nutrients, it follows that the speed at which micro-organisms carry out metabolic reactions is very much greater than the speed found in higher organisms, and this relatively high speed of metabolism helps to make micro-organisms valuable agents in industrial processes.

One of the best, and certainly the most often quoted, example