Fermentation Microbiology and Biotechnology

Edited by
E.M.T. El-Mansi • C.F.A. Bryce
A.L. Demain • A.R. Allman

CRC Taylor & Francis
Taylor & Francis Group

Contents

Chapter 1 Fermentation Microbiology and Biotechnology: An Historical Perspective 1
E.M.T. El-Mansi, C.F.A. Bryce, Brian S. Hartley and Arnold L. Demain

1.1 Fermentation: An ancient tradition 1
1.2 The rise of fermentation microbiology 1
1.3 Developments in metabolic and biochemical engineering 4
1.4 Discovery of antibiotics and genetic engineering 6
1.5 The rise and fall of single cell protein 7
1.6 Fermentation biotechnology and the production of amino acids 7
1.7 Impact of functional genomics, proteomics, metabolomics and bio-informatics on the scope and future prospects of fermentation microbiology and biotechnology 8
References 9

Chapter 2 Microbiology of Industrial Fermentation 11
E.M.T. El-Mansi and F. Bruce Ward

2.1 Introduction 11
2.2 Chemical synthesis of bacterial protoplasm/biomass 12
2.2.1 Central and intermediary metabolism 12
2.2.2 Anaplerotic pathways 14
2.2.3 Polymerization and assembly 16
2.2.4 Biomass formations 16
2.3 The growth cycle 16
2.3.1 The lag phase 18
2.3.2 The exponential phase 20
2.3.3 Stationary phase and cell death 24
2.3.4 Maintenance and survival 26
2.4 Diauxic growth 29
2.5 Growth yield in relation to carbon and energy contents of growth substrates 30
2.6 Fermentation balances 31
2.6.1 Carbon balance 31
2.6.2 Redox balance 32

2.7 Efficiency of central metabolism 32
2.7.1 Futile cycling and efficiency of central metabolism 32
2.7.2 Metabolite excretion and efficiency of central metabolism 34
2.8 Continuous cultivation of micro-organisms 35
2.8.1 Types of continuous cultures 35
2.9 Current advances and innovations in the fermentation and pharmaceutical industry 36
2.9.1 The "quiescent cell factory": A novel approach 36
2.10 Microbial fermentations and the production of biopharmaceuticals 37
2.10.1 Hyaluronic acid synthesis: a case study 37
Summary 42
References 43
Suggested reading 45

Chapter 3 Fermentation Kinetics 47
Jens Nielsen

3.1 Introduction 47
3.2 Framework for kinetic models 49
3.2.1 Stoichiometry 51
3.2.2 Reaction rates 53
3.2.3 Yield coefficients and linear rate equations 55
3.2.4 The black box model 63
Example 3b: Elemental balances in a simple black box model 67
3.3 Mass balances for bioreactors 68
3.3.1 Dynamic mass balances 69
3.3.2 The batch reactor 73
3.3.3 The chemostat 74
3.3.4 The fed-batch reactor 76
3.4 Kinetic models 77
3.4.1 The degree of model complexity 78
3.4.2 Unstructured models 79
Example 3c: The Monod model 81
3.4.3 Compartment models 84
Example 3d: A two-compartment model 85
3.4.4 Single-cell models 88
3.4.5 Molecular mechanistic models 89
3.5 Population models 91
3.5.1 Morphologically structured models 92
3.5.2 Population balance equations 93
Example 3e: Age distribution model 94
Summary 95
References 95

Chapter 4 Microbial Synthesis of Primary Metabolites: Current Advances and Future Prospects 99
A.L. Demain and Sergio Sanchez

4.1 Introduction 99
4.2 Control of primary metabolism 100
4.2.1 Induction 100
4.2.2 Catabolite repression 100
4.2.3 Nitrogen source regulation (NSR) 101
4.2.4 Phosphorus source regulation 101
4.2.5 Sulfur source regulation 102
4.2.6 Feedback regulation 103
4.2.7 Additional types of regulation 103
4.3 Approaches to strain improvements 104
4.4 Production of primary metabolites 106
4.4.1 Amino acids production 106
4.4.2 Production processes for purines and pyrimidines, their nucleosides and nucleotides 117
4.4.3 Production processes for vitamins 120
4.4.4 Production processes for organic acids 122
4.4.5 Production of ethanol and related compounds 126
Summary 129
References and suggested reading 129

Chapter 5 Microbial Synthesis of Secondary Metabolites and Strain Improvement 131
Iain S. Hunter

5.1 Introduction 131
5.2 The economics and scale of microbial product fermentations 132
5.3 Different products need different fermentation processes 133
5.4 Fed-batch culture; the paradigm for many efficient microbial processes 135
5.4.1 Nutrient limitation and the onset of secondary metabolite formation 136
5.4.2 The role of quorum sensing and extracellular signals in the initiation of secondary metabolism and morphological differentiation in actinomycetes 138
5.4.3 Positive activators of antibiotic expression 139
5.5 Tactical issues for strain improvement programs 139
5.6 Strain improvement: The random, empirical approach 141
5.7 Strain improvement: The power of recombination in "strain construction" 143
5.8 Directed screening for mutants with altered metabolism 145

5.9 Recombinant DNA approaches to strain improvement for low- and medium-value products 150
5.10 Strain improvement for high-value recombinant products 153
Summary 156
References 156

Chapter 6 Metabolic Analysis and Optimization of Microbial and Animal Cell Bioprocesses 159
David M. Mousdale

6.1 Metabolic analysis in the era of genomics, proteomics, and metabolomics 159
6.2 Secondary product fermentations 162
6.2.1 Clavulanic acid 162
6.2.2 Demethyl-chlortetracyclines 163
6.2.3 A novel cyclic octodepsipeptide 165
6.2.4 Zeaxanthin 166
6.3 Microbial production of industrial enzymes 168
6.3.1 Metabolic problems and perspectives 168
6.3.2 Protease fermentations 169
6.3.3 Cellulase fermentations 172
6.3.4 Solid-state fermentations – the renaissance of an old technology? 173
6.4 Animal cells and recombinant protein production in bioreactors 174
6.4.1 Production of biopharmaceuticals: Microbes or animal cells? 174
6.4.2 Yeasts in stirred-tank fermentors 174
6.4.2 Animal cells in bioreactors 176
6.5 Future prospects 180
Summary 182
References 182

Chapter 7 Flux Control Analysis: Basic Principles and Industrial Applications 187
E.M.T. El-Mansi and Gregory Stephanopoulos

7.1 Introduction: traditional versus modern concepts 187
7.2 Flux control analysis: basic principles 189
7.2.1 The flux control coefficient 189
7.2.2 The summation theorem 191
7.2.3 Elasticity coefficient 192
7.2.4 The connectivity theorem 193
7.2.5 Response coefficients 194
7.3 Control of carbon flux at the junction of isocitrate in central metabolism during growth of *Escherichia coli* on acetate: a case study 194
7.3.1 The model 196
7.4 Modeling using other computer programs 202

7.4.1 Modeling of the partition of isocitrate flux with Gepasi 202
7.5 Strategies for manipulating carbon fluxes en route to product formation in intermediary metabolism 204
7.5.1 Validity of the concept of the "rate-limiting" step as an approach to increasing flux to product formation 204
7.5.2 Modulation of carbon flux en route to product formation 205
7.6 Conversion of feedstock to biomass and desirable end products 208
7.6.1 Stoichiometric analysis 208
7.6.2 Formulation of metabolic flux models/charts 209
7.6.3 Applications of flux distribution analysis 209
Summary 212
Acknowledgment 214
References 214

Chapter 8 Enzyme and Co-factor Engineering and Their Applications in the Pharmaceutical and Fermentation Industries 217
George N. Bennett and K.-Y. San

8.1 Introduction 217
8.2 Types of major industrial enzymes and desired modifications 218
8.2.1 Targets for enzyme engineering 218
8.2.2 Hydrolytic enzymes 219
8.2.3 Specialty enzymes 220
8.2.4 Alteration of physical parameters of enzymes for process applications 221
8.3 Summary of methods in enzyme engineering 222
8.3.1 Site-specific mutagenesis 222
8.3.2 Cassette mutagenesis 222
8.3.3 3-D structure and specific mutations 224
8.3.4 Random "directed evolution" methods 225
8.4 Modification of pharmaceutical properties of protein agents 226
8.5 Modification of enzymes for *in vivo* biosynthetic processes 229
8.6 Co-factor engineering 230
8.6.1 NADH vs. NADPH specificity of enzymes 231
8.6.2 Manipulation of NADH *in vivo* 232
8.6.3 CoA compounds 234
Summary 236
References 237

Chapter 9 Application of Metabolic Engineering to the Conversion of Renewable Resources to Fuels and Fine Chemicals: Current Advances and Future Prospects 249
Aristos A. Aristidou

9.1 Introduction 249
9.2 Pentose fermentation 258
9.3 Genetically engineered bacteria 259
9.3.1 *Escherichia coli* 259
9.3.2 *Klebsiella oxytoca* 264
9.3.3 *Zymomonas mobilis* 265
9.4 Genetically engineered yeast 268
9.4.1 *Saccharomyces cerevisiae* 268
9.4.2 *Pichia stipitis* 273
9.4.3 *Pichia pastoris* 275
9.4.4 Fungal xylose isomerase in yeast 275
9.5 Microbes producing ethanol from lignocellulose 277
Conclusions 278
References 279
Summary 279

Chapter 10 Cell Immobilization and Its Applications in Biotechnology: Current Trends and Future Prospects 287
Ronnie Willaert

10.1 Introduction 287
10.2 Immobilized cell systems 288
10.2.1 Surface attachment of cells 288
10 2.2 Entrapment within porous matrices 290
10.2.2.1 Hydrogel entrapment 291
10.2.2 2 Preformed support materials 303
10.2.3 Containment behind a barrier 304
10.2.3.1 Micro-encapsulation 307
10.2.3.2 Cell immobilization using membranes 309
10.2.4 Self-aggregation of cells 313
10.3 Design of immobilized cell reactors 315
10.3.1 Mass transport phenomena in immobilized cell systems 315
10.3.1.1 Diffusion coefficient 315
10.3.1.2 Diffusion in immobilized cell systems 316
10.3.1.3 External mass transfer 317
10.3.2 Reaction and diffusion in immobilized cell systems 319
10.3.2.1 Reaction-diffusion models 319
10.3.3 Bioreactor design 324
10.4 Physiology of immobilized microbial cells 325
10.4.1 Bacterial cells 328
10.4.2 Fungal cells 332

10.5 Beer production using immobilized cell technology: A case study 333
10 5.1 Flavor maturation of green beer 333
10.5.2 Production of alcohol-free or low-alcohol beer 337
10.5.3 Continuous main fermentation 337
Summary 340
Acknowledgments 341
References 341

Chapter 11 Biosensors in Bioprocess Monitoring and Control: Current Trends and Future Prospects 363
Chris E. French and Marco F. Cardosi

11.1 Introduction 363
11.2 Biosensors in process monitoring 363
11.3 Overview of transduction methods 366
11.4 Catalytic biosensors: Enzymes as biological sensing elements 368
11.5 Affinity biosensors: Antibodies as biological detection elements 370
11.6 Immobilization of the biological recognition element 373
11.7 Amperometric biosensors based on redox enzymes 375
11.8 Potentiometric biosensors and enzyme field effect transistor (ENFET) 380
11.9 Thermal biosensors 382
11.10 Optical biosensors based on redox enzymes 383
11.11 Indirect affinity sensors: Optical and electrical biosensors based on antibodies 384
11.12 Direct affinity detection using surface plasmon resonance and piezoelectric biosensors 385
11.13 Amperometric glucose biosensors for blood glucose monitoring: A case study 388
11.13.1 Home blood glucose monitoring: The glucose meter 391
11.13.2 Enzymes used in glucose biosensors 392
11.13.3 Mediated electrochemistry 396
11.13.4 The electrochemical measurement 396
References 399
Summary 399

Chapter 12 Fermentors: Design, Operation, and Applications 407
A.R. Allman

12.1 Batch culture fermentation 407
12.2 The main components of a fermentor and their uses 407
12.3 Component parts of a typical vessel 408
12.4 Peripheral parts and accessories 409

	12.4.1	Reagent pumps	409
	12.4.2	Medium feed pumps and reservoir bottles	410
	12.4.3	Rotameter/gas supply	410
	12.4.4	Sampling device	411
12.5	Alternative vessel designs		411
	12.5.1	Air lift	411
	12.5.2	Fluidized bed, immobilized and solid-state systems	412
	12.5.3	Hollow fiber	413
	12.5.4	*In situ* sterilizable fermentors	413
	12.5.5	Containment	414
12.6	Different types of instrumentation		414
	12.6.1	Digital controllers — embedded microprocessor	415
	12.6.2	Digital controllers — process controllers	415
	12.6.3	Digital controllers — direct computer control	415
12.7	Common measurement and control systems		416
	12.7.1	Speed control	416
	12.7.2	Temperature control	416
	12.7.3	Control of gas supply	418
	12.7.4	Control of pH	419
	12.7.5	Control of dissolved oxygen	420
	12.7.6	Antifoam control	421
12.8	Additional sensors		422
	12.8.1	Redox	423
	12.8.2	Air flow	424
	12.8.3	Weight	424
	12.8.4	Pressure	424
	12.8.5	On-line measurement of biomass	425
12.9	Simple continuous culture		426
12.10	Additional accessories and peripherals		426
	12.10.1	Feed pumps	426
	12.10.2	Exit gas analysis	429
	12.10.3	Substrate sensors	431
12.11	Fermentor preparation and use		432
	12.11.1	Disassembly of the vessel	432
	12.11.2	Cleaning	432
	12.11.3	Preparations for autoclaving	433
	12.11.4	Autoclaving	435
	12.11.5	Set-up following autoclaving	435
	12.11.6	Inoculation of a fermentor vessel	437
	12.11.7	Sampling from a fermentor vessel	437
	12.11.8	Routine maintenance of fermentor components	439
12.12	Major types of organisms used in fermentation		441
	12.12.1	Bacteria/yeast/fungi	441
	12.12.2	Plant cells	443
	12.12.3	Mammalian cell culture	443

12.12.4 Algae 444
12.13 Subfermentor systems — a new approach 445
12.13.1 Parallel small fermentor systems 446
12.13.2 Simplified fermenter systems 446
12.14 Solutions to common problems in fermentation 447
12.14.1 General hardware problems 447
12.14.2 Contamination problems 448
12.14.3 Poor growth of the microbe 448
Summary 449
References and suggested reading 450

Chapter 13 Control of Fermentations: An Industrial Perspective 451
Craig J.L. Gershater

13.1 Requirement for control 451
13.1.1 Microbial growth 451
13.1.2 Nature of control 452
13.1.3 Control loop strategy 452
13.2 Sensors 453
13.2.1 Historical perspective 453
13.2.2 Typical fermentation sensors 454
13.2.3 Control action 456
13.3 Controllers 457
13.3.1 Types of control 457
13.3.2 Control algorithms 458
13.4 Design of a fermentation control system 460
13.4.1 Control system objectives 460
13.4.2 Fermentation computer control system architecture 463
13.4.3 Fermentation plant safety 465
13.5 Fermentor control specification 465
13.5.1 Specifying sequence control 465
13.5.2 Fermentation unit operations 465
13.5.3 Vessel states 467
13.5.4 Sequence logic 468
13.5.5 Flow charting 468
13.6 Control of incubation 472
13.6.1 Specification for incubation control 473
13.7 Advanced incubation control 479
13.7.1 Fermentation profiles 479
13.7.2 Event-tracking control 480
13.7.3 Boolean control and rule generation 484
13.7.4 Summary of event and nonstable set-point control 486
13.8 Other advanced fermentation control options 486
13.8.1 Knowledge-based systems (KBSs) 487
13.8.2 Artificial neural networks (ANNs) 487
13.8.3 Genetic algorithms (GAs) 487
13.8.4 Modeling 488

Editorial update 488
13.9 Recent trends in fermentation Control 488
13.9.1 New sensor technology 488
13.9.2 Expansion of the capability of DDC instrumentation 489
13.9.3 Use of common communication protocols 490
Acknowledgments 491
Suggested reading 491
Summary 491

Chapter 14 Modeling, Software Sensors, Control, and Supervision of Fermentation Processes 493
Boutaieb Dahhou, Gilles Roux and Y. Nakkabi

14.1 Introduction 493
14.2 The model system 494
14.2.1 Off-line measurements 495
14.2.2 On-line measurements 496
14.3 Modeling 496
14.3.1 Unstructured models 497
14.3.2 Behavioral models 499
14.4 Adaptive techniques 503
14.4.1 Estimation and software sensors 504
14.4.2 Control 506
14.5 Supervision for process control 508
14.5.1 Classification 509
14.5.2 Fault detection and isolation (FDI) 513
14.6 Conclusions 518
Summary 520
References 520

Appendix: Suppliers List 523
Instrumentation, Sensors and Software 523
Fermentation Equipment 524

Index 525