

Contents

Chapter 1 Fermentation Microbiology and Biotechnology: An Historical Perspective	1
<i>E.M.T. El-Mansi, C.F.A. Bryce, Brian S. Hartley and Arnold L. Demain</i>	
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
<i>E.M.T. El-Mansi and F. Bruce Ward</i>	
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
	<i>Jens Nielsen</i>	
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 Example 3b: Elemental balances in a simple black box model	63
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 Example 3c: The Monod model	81
3.4.3	Compartment models 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 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
<i>David M. Mousdale</i>		
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
<i>E.M.T. El-Mansi and Gregory Stephanopoulos</i>		
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 <i>Escherichia coli</i> 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 <i>in vivo</i> 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 <i>in vivo</i>	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	<i>Escherichia coli</i>	259
9.3.2	<i>Klebsiella oxytoca</i>	264
9.3.3	<i>Zymomonas mobilis</i>	265
9.4	Genetically engineered yeast	268
9.4.1	<i>Saccharomyces cerevisiae</i>	268
9.4.2	<i>Pichia stipitis</i>	273
9.4.3	<i>Pichia pastoris</i>	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

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

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	<i>In situ</i> 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
	<i>Craig J.L. Gershater</i>	
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
<i>Boutaieb Dahhou, Gilles Roux and Y. Nakkabi</i>	
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
 <i>Index</i>	 525