

Contents

Preface	<i>xiii</i>
Acknowledgments	<i>xxi</i>
Nomenclature for Part I	<i>xxiii</i>
List of Simulation Examples	<i>xxviii</i>

Part I Principles of Bioreactor Modeling 1

1	Modeling Principles	3
1.1	Fundamentals of Modeling	3
1.1.1	Use of Models for Understanding, Design, and Optimization of Bioreactors	3
1.1.2	General Aspects of the Modeling Approach	4
1.1.3	General Modeling Procedure	6
1.1.4	Simulation Tools	8
1.1.5	Teaching Applications	8
1.2	Development and Meaning of Dynamic Differential Balances	9
1.2.1	Derivation of a Balance Equation Using Rates	11
1.2.2	Computer Solution	12
1.3	Formulation of Mass Balance Equations	13
1.3.1	Types of Mass Balance Equations	13
1.3.2	Balancing Procedure	15
1.3.2.1	Case A: Continuous Stirred Tank Bioreactor	15
1.3.2.2	Case B: Tubular Reactor	16
1.3.2.3	Case C: River with Eddy Current	16
1.3.3	Total Mass Balances	23
1.3.4	Component Balances for Reacting Systems	24
1.3.4.1	Case A: Constant Volume Continuous Stirred Tank Reactor	25
1.3.4.2	Case B: Semicontinuous Reactor with Volume Change	26
1.3.4.3	Case C: Steady-state Oxygen Balancing in Fermentation	27
1.3.4.4	Case D: Inert Gas Balance to Calculate Flow Rates	28
1.4	Additional Relationships	29
1.4.1	Stoichiometry and Metabolite and Elemental Balancing	29

1.4.1.1	Simple Stoichiometry	29
1.4.1.2	Metabolic Network Stoichiometry: Metabolite Balancing	30
1.4.1.3	Elemental Balancing	31
1.4.2	Yield Coefficients	33
1.4.2.1	Mass Yield Coefficients	33
1.4.2.2	Selectivity	34
1.4.2.3	Energy Yield Coefficients	34
1.5	Thermodynamics and Equilibrium Relationships	35
1.5.1	Reaction Enthalpy	35
1.5.2	Chemical Equilibrium	35
1.5.3	Receptor Binding	35
1.5.4	Case A: Calculation of pH with an Ion Charge Balance	36
1.6	Energy Balancing for Bioreactors	38
1.6.1	Accumulation Term	39
1.6.2	Flow Term	39
1.6.3	Water Evaporation Term	40
1.6.4	Heat Transfer Term	41
1.6.5	Reaction Heat Term	41
1.6.6	Case B: Determining Heat Production Rate of a Batch Fermentation	42
1.6.7	Case C: Determining Heat Transfer Area or Cooling Water Temperature	42
1.7	Time Constants	43
1.7.1	Derivation from Differential Equations	44
1.7.2	Derivation from Capacity and Rate	45
2	Basic Bioreactor Concepts	47
2.1	Information for Bioreactor Modeling	47
2.2	Bioreactor Operation	48
2.2.1	Batch Operation	48
2.2.2	Semicontinuous or Fed-batch Operation	50
2.2.3	Continuous Operation	51
2.2.4	Summary and Comparison of Bioreactors	54
3	Biological Kinetics	57
3.1	Enzyme Kinetics	58
3.1.1	Reaction Equilibrium	58
3.1.2	Michaelis–Menten Equation	58
3.1.3	Other Enzyme Kinetic Models	61
3.1.3.1	Double Michaelis–Menten Kinetics	62
3.1.3.2	Inhibition	62
3.1.3.3	Substrate Inhibition	63
3.1.3.4	Allosteric Kinetics	64
3.1.3.5	Temperature and pH Influence	64
3.1.4	Enzyme Deactivation	65
3.2	Simple Microbial Kinetics	65

3.2.1	Basic Growth Kinetics	65
3.2.2	Cell Death and Sterilization	67
3.2.3	Specific Rates	67
3.2.4	Monod Growth Kinetics	68
3.2.5	Substrate Inhibition of Growth	69
3.2.6	Product Inhibition	70
3.2.7	Other Expressions for Specific Growth Rate	70
3.2.8	Substrate Uptake Kinetics	71
3.2.9	Substrate Uptake in Wastewater Systems	72
3.2.10	Product Formation	72
3.3	Interacting (Micro-)organisms	72
3.3.1	Case A: Modeling of Mutualism Kinetics	75
3.3.2	Case B: Kinetics of Anaerobic Degradation	76
3.4	Structured Kinetic Models	77
3.4.1	Types of Structured Kinetic Models	77
3.4.2	Examples of Simple Structured Models	78
3.4.2.1	Case C: Modeling Growth and Synthesis of Poly- β -hydroxybutyric Acid (PHB)	79
3.4.2.2	Case D: Modeling of Sustained Oscillations in Continuous Baker's Yeast Culture	80
3.4.2.3	Case E: Growth and Product Formation of an Oxygen-Sensitive <i>Bacillus subtilis</i> Culture	81
3.4.3	Modeling of Metabolic Networks	84
3.4.3.1	Case F: Dynamic Kinetic Model for Describing Metabolic Fluxes Secondary Metabolite Synthesis Pathway	85
3.4.3.2	Case G: Modeling the Dynamics of Mammalian Cell Cultivation in Fed-batch Cultures	88
4	Basic Bioreactor Modeling	91
4.1	General Balances for Tank-type Biological Reactors	91
4.1.1	The Batch Fermenter	92
4.1.2	The Chemostat	93
4.1.3	The Fed-batch Fermenter	96
4.1.4	Biomass Productivity	97
4.1.5	Case A: Continuous Fermentation with Biomass Recycle	98
4.1.6	Case B: Enzymatic Tanks-in-series Bioreactor System	100
4.2	Modeling Tubular Plug Flow Bioreactors	102
4.2.1	Steady-state Balancing	102
4.2.2	Unsteady-state Balancing for Tubular Bioreactors	103
5	Mass Transfer	105
5.1	Mass Transfer in Biological Reactors	105
5.1.1	Gas Absorption with Bioreaction in Liquid Phase	105
5.1.2	Liquid-Liquid Extraction with Bioreaction in One Phase	105
5.1.3	Surface Biocatalysis	105

5.1.4	Diffusion and Reaction in Porous Biocatalyst	106
5.2	Interphase Gas–Liquid Mass Transfer	106
5.3	General Oxygen Balances for Gas–Liquid Transfer	109
5.3.1	Application of Oxygen Balances	111
5.3.1.1	Case A: Steady-state Gas Balance to Determine the Biological Uptake Rate	111
5.3.1.2	Case B: Determination of $k_L a$ Using a Chemical or Biochemical Reaction Consuming Oxygen	111
5.3.1.3	Case C: Determination of $k_L a$ by a Dynamic Method	112
5.3.1.4	Case D: Determination of Oxygen Uptake Rates by a Dynamic Method	113
5.3.1.5	Case E: Steady-state Liquid Balancing to Determine Oxygen Uptake Rate	114
5.3.1.6	Case F: Steady-state Deoxygenated Feed Method for $k_L a$	115
5.3.1.7	Case G: Biological Oxidation in an Aerated Tank	115
5.3.1.8	Case H: Modeling Nitrification in a Fluidized Bed Biofilm Reactor	117
5.4	Models for Oxygen Transfer in Large-scale Bioreactors	120
5.4.1	Case A: Model for Oxygen Gradients in a Bubble Column Bioreactor	122
5.4.2	Case B: Model for a Multiple Impeller Fermenter	123
6	Diffusion and Biological Reaction in Immobilized Biocatalyst Systems	127
6.1	External Mass Transfer	128
6.2	Internal Diffusion and Reaction Within Biocatalysts	130
6.2.1	Derivation of Finite Difference Model for Diffusion–Reaction Flat Plate Systems	132
6.2.2	Finite Difference Model for Diffusion–Reaction in a Sphere	135
6.2.3	Dimensionless Parameters from Diffusion–Reaction Models	136
6.2.4	The Effectiveness Factor Concept	137
6.2.5	Case A: Estimation of Oxygen Diffusion Effects in a Biofilm	138
6.2.6	Case B: Complex Diffusion–Reaction Processes (Biofilm Nitrification)	138
7	Automatic Bioprocess Control Fundamentals	143
7.1	Elements of Feedback Control	144
7.2	Measurement of Process Variables	144
7.2.1	Sensors Used in Biotechnology	145
7.2.2	Calculated Measured Variables	146
7.2.3	Dynamic Characteristics of Measurement	146
7.3	Types of Controller Action	147
7.3.1	On–Off Control	147
7.3.2	Proportional (P) Controller	147
7.3.3	Proportional–Integral (PI) Controller	148
7.3.4	Proportional–Integral–Derivative (PID) Controller	149

7.4	Controller Tuning	150
7.4.1	Trial and Error Method	151
7.4.2	Ziegler–Nichols Method	151
7.4.3	Ultimate Gain Method	152
7.4.4	Error Integrals for Characterization of Controller Performance	152
7.5	Advanced Control Strategies	153
7.5.1	Cascade Control	153
7.5.2	Feed-forward Control	153
7.5.3	Adaptive Control	154
7.5.4	Other Types of Advanced Control	155
7.6	Application Strategies of Bioprocess Control	155
8	Basic Cell and Bioreactor Models	159
8.1	Basic Cell Balances	160
8.2	The Link of the Cell Balances to a Bioreactor	162
8.2.1	Continuous Well-Mixed Stirred-tank Bioreactor (Chemostat)	162
8.2.2	Batch Bioreactor	164
8.2.3	Case A: Checking for Metabolic Steady State in a Batch Culture	164
8.2.4	Case B: Compartmented Cell and Bioreactor Modeling	165
8.3	Organism Modeling	168
8.3.1	Case C: Bioreactor and Human-body Model for Toxicity Prediction	169
	References Part I and Recommended Textbooks and References for Further Reading	173
	Part II Dynamic Bioprocess Modeling and Simulation Examples Using the Berkeley Madonna Simulation Language	187
9	Dynamic Bioprocess Modeling Examples	189
9.1	Modeling a Roman Fountain	190
9.2	Modeling a Lake	191
9.3	Modeling a Mammalian Cell Recirculation Reactor with External Aeration	192
9.4	Modeling Protein Synthesis and Secretion in a Eukaryotic Cell	193
9.5	Modeling a Liver Sinusoid	194
10	Simulation Examples of Biological Reaction Processes Using Berkeley Madonna	197
10.1	Introductory Simulation Examples	199
10.1.1	Batch Fermentation (BATFERM)	199
10.1.2	Chemostat Fermentation (CHEMO)	204
10.1.3	Fed-batch Fermentation (FEDBAT)	208
10.1.4	Introductory Exercises in Bioreactor Model Building	212

- 10.2 Batch Reactors 229**
 - 10.2.1 Kinetics of Enzyme Action (MMKINET) 229
 - 10.2.2 Lineweaver–Burk Plot (LINEWEAV) 231
 - 10.2.3 Oligosaccharide Production in Enzymatic Lactose Hydrolysis (OLIGO) 234
 - 10.2.4 Batch Heat Sterilization (BATSTER) 237
 - 10.2.5 Growth of the Coronavirus (CORONADYN) 242

- 10.3 Fed-batch Reactors 247**
 - 10.3.1 Variable Volume Fermentation (VARVOL and VARVOLD) 247
 - 10.3.2 Penicillin Fermentation Using Elemental Balancing (PENFERM) 252
 - 10.3.3 Ethanol Fed-batch Diauxic Fermentation (ETHFERM) 260
 - 10.3.4 Repeated Fed-batch Culture (REPFED) 264
 - 10.3.5 Repeated Medium Replacement Culture (REPLCUL) 267
 - 10.3.6 Penicillin Production in a Fed-batch Fermenter (PENOXY) 270

- 10.4 Continuous Reactors 275**
 - 10.4.1 Steady-state Chemostat (CHEMOSTA) 275
 - 10.4.2 Continuous Culture with Inhibitory Substrate (CONINHIB) 278
 - 10.4.3 Nitrification in Activated Sludge Process (ACTNITR) 283
 - 10.4.4 Tubular Enzyme Reactor (ENZTUBE) 287
 - 10.4.5 Dual Substrate Limitation (DUAL) 290
 - 10.4.6 Two-stage Chemostat with Additional Stream (TWOSTAGE) 294
 - 10.4.7 Two-stage Culture with Product Inhibition (STAGED) 298
 - 10.4.8 Fluidized Bed Recycle Reactor (FBR) 301
 - 10.4.9 Nitrification in a Fluidized Bed Reactor (NITBED) 305
 - 10.4.10 Continuous Enzymatic Reactor (ENZCON) 310
 - 10.4.11 Reactor Cascade with Deactivating Enzyme (DEACTENZ) 313
 - 10.4.12 Continuous Production of PHB in a Two-tank Reactor Process (PHBTWO) 317
 - 10.4.13 Dichloromethane in a Biofilm Fluidized Sand Bed (DCMDEG) 321

- 10.5 Oxygen Uptake Systems 329**
 - 10.5.1 Aeration of a Tank Reactor for Enzymatic Oxidation (OXENZ) 329
 - 10.5.2 Gas and Liquid Oxygen Dynamics in a Continuous Fermenter (INHIB) 332
 - 10.5.3 Batch Nitrification with Oxygen Transfer (NITRIF) 337
 - 10.5.4 Oxygen Uptake and Aeration Dynamics (OXDYN) 340
 - 10.5.5 KLADYN, KLAFIT, and ELECTFIT: Dynamic Oxygen Electrode Method for K_La 343
 - 10.5.6 Biofiltration Column for Removing Two Inhibitory Substrates (BIOFILTDYN) 349
 - 10.5.7 Optical Sensing of Dissolved Oxygen in Microtiter Plates (TITERDYN and TITERBIO) 354

- 10.6 Diffusion Systems 361**
 - 10.6.1 Double Substrate Biofilm Reaction (BIOFILM) 361
 - 10.6.2 Steady-state Split Boundary Solution (ENZSPLIT) 366
 - 10.6.3 Dynamic Porous Diffusion and Reaction (ENZDYN) 369
 - 10.6.4 Oxygen Diffusion in Animal Cell Sphere (CELLDIFFBEAD) 374
 - 10.6.5 Oxygen Diffusion to a Single Cell or Cell Aggregate (CELLDIFFCYL) 378
 - 10.6.6 Immobilized Biofilm in a Nitrification Column System (NITBEDFILM) 385

- 10.7 Controlled Reactors 393**
 - 10.7.1 Feedback Control of a Water Heater (TEMPCONT) 393
 - 10.7.2 Temperature Control of Fermentation (FERMTEMP) 397
 - 10.7.3 Turbidostat Response (TURBCON) 402
 - 10.7.4 Control of a Continuous Bioreactor with Inhibitory Substrate (CONTCON) 405
 - 10.7.5 Adaptive Control of Dissolved Oxygen at Low Levels in Batch Culture (ADAPTOXCONT) 410

- 10.8 Membrane and Cell Retention Reactors 419**
 - 10.8.1 Cell Retention Membrane Reactor (MEMINH) 419
 - 10.8.2 Fermentation with Pervaporation (PERVAPSUB) 422
 - 10.8.3 Two-stage Fermentor with Cell Recycle for Continuous Production of Lactic Acid (LACMEMRECYC) 430
 - 10.8.4 Tubular Hollow Fiber Enzyme Reactor Module for Lactose Hydrolysis (LACREACT) 435
 - 10.8.5 Immobilized Animal Cells in a Fluidized Bed Reactor (ANIMALIMMOB) 441

- 10.9 Multi-organism Systems 447**
 - 10.9.1 Two Bacteria with Opposite Substrate Preferences (COMMENSA) 447
 - 10.9.2 Competitive Assimilation and Commensalism (COMPASM) 452
 - 10.9.3 Stability of Recombinant Microorganisms (PLASMID) 456
 - 10.9.4 Predator–Prey Population Dynamics (MIXPOP) 461
 - 10.9.5 Competition Between Organisms (TWOONE) 465
 - 10.9.6 Competition Between Two Microorganisms for an Inhibitory Substrate in a Biofilm (FILMPOP) 468
 - 10.9.7 Model for Anaerobic Reactor Activity Measurement (ANAEMEAS) 473
 - 10.9.8 Dynamics of an Epidemic Using the SIR Model (SIRDYN and SIRDYNDIM) 479

- 10.10 Structured and Metabolic Network Models 487**
 - 10.10.1 Oscillations in Continuous Yeast Culture (YEASTOSC) 487
 - 10.10.2 Structured Model for PHB Production (PHB) 492
 - 10.10.3 Mammalian Cell Cycle Control (MAMMCELLCYCLE) 496

- 10.10.4 Metabolic Dynamics in Secondary Metabolite Fluxes in Potato Tubers (POTATO) 500
- 10.10.5 Structured Model of the Production of Acetoin and Butanediol (SUBTILIS) 505
- 10.10.6 Dynamics of Cultivation of CHO for the Production of Monoclonal Antibodies (CHOMAB) 509

Appendix A Using the Berkeley Madonna Language and Accessing the Simulation Examples: A Short Guide 519

- A.1. Computer Requirements 519
- A.2. Downloading Simulation Examples and the Berkeley Madonna Program for this Book 519
- A.3. Running Programs 520

Index 521