

Edited by
Ganapathy Subramanian

Continuous Biomanufacturing

Innovative Technologies
and Methods



Edited by
Ganapathy Subramanian

**Continuous
Biomanufacturing**

Continuous Biomanufacturing

Innovative Technologies and Methods

Edited by Ganapathy Subramanian

WILEY-VCH
Verlag GmbH & Co. KGaA

Editor

Prof. Dr. Ganapathy Subramanian
44 Oaken Grove
SL6 6HH Maidenhead, Berkshire
United Kingdom

Cover

Pictures being used: Coagulation factor VIII protein rendering © Molekuul.be; Vitaminpills © cst21; Measurement device containing closed vials © eGraphia.

■ All books published by **Wiley-VCH** are carefully produced. Nevertheless, authors, editors, and publisher do not warrant the information contained in these books, including this book, to be free of errors. Readers are advised to keep in mind that statements, data, illustrations, procedural details or other items may inadvertently be inaccurate.

Library of Congress Card No.:
applied for

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library.

Bibliographic information published by the Deutsche Nationalbibliothek

The Deutsche Nationalbibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data are available on the Internet at <<http://dnb.d-nb.de/>>.

© 2018 Wiley-VCH Verlag GmbH & Co. KGaA, Boschstr. 12, 69469 Weinheim, Germany

All rights reserved (including those of translation into other languages). No part of this book may be reproduced in any form – by photoprinting, microfilm, or any other means – nor transmitted or translated into a machine language without written permission from the publishers. Registered names, trademarks, etc. used in this book, even when not specifically marked as such, are not to be considered unprotected by law.

Print ISBN: 978-3-527-34063-7

ePDF ISBN: 978-3-527-69989-6

ePub ISBN: 978-3-527-69991-9

Mobi ISBN: 978-3-527-69992-6

oBook ISBN: 978-3-527-69990-2

Cover Design Bluesea Design, MacLeese Lake, Canada
Typesetting Thomson Digital, Noida, India

Printing and Binding

Printed on acid-free paper

10 9 8 7 6 5 4 3 2 1

Contents

List of Contributors *xix*

Part One: Overview of State-of-the-Art Technologies and Challenges 1

- 1 **Continuous Bioprocess Development: Methods for Control and Characterization of the Biological System 3**
Peter Neubauer and M. Nicolas Cruz-Bournazou
- 1.1 Proposed Advantages of Continuous Bioprocessing 3
 - 1.1.1 Introduction 3
- 1.2 Special Challenges for Continuous Bioprocesses 5
 - 1.2.1 The Biological System in Continuous Biomanufacturing 5
 - 1.2.2 Inherent Changes in the Microbial System – Problem of Evolution 6
 - 1.2.3 Lack of Process Information 7
 - 1.2.3.1 Models-Based Process Development and Control for Continuous Processes 8
 - 1.2.3.2 Engineering Approach to Complex Systems 8
 - 1.2.4 Limited Control Strategies 9
 - 1.2.4.1 Traditional Control Strategies for Continuous Cultures 9
- 1.3 Changes Required to Integrate Continuous Processes in Biotech 11
 - 1.3.1 A Better Physiological Understanding of the Organisms and Their Responses on the Reactor Environment 11
 - 1.3.1.1 Model Complexity 11
 - 1.3.1.2 Models 12
 - 1.3.2 Model-Based Process Monitoring 13
 - 1.3.3 Implementation of Model Predictive Control 14
 - 1.3.3.1 Model-Based Control 14
- 1.4 Role of Iterative Process Development to Push Continuous Processes in Biotech 14
 - 1.4.1 Methods for Development of Continuous Processes 14
 - 1.4.1.1 Alternative: Fed-Batch as a System to Simulate Quasi Steady-State Conditions 16

- 1.4.2 Mimicking Industrial Scale Conditions in the Lab: Continuous-Like Experiments 17
 - 1.4.2.1 A Simple Model for Continuous Processes 17
 - 1.4.2.2 Continuous-Like Fed-Batch Cultivations 18
- 1.4.3 Fast and Parallel Experimental Approaches with High Information Content 20
 - 1.4.3.1 Computer-Aided Operation of Robotic Facilities 20
 - 1.4.3.2 Model Building and Experimental Validation 21
- 1.5 Conclusions 22
- References 22

2 Tools Enabling Continuous and Integrated Upstream and Downstream Processes in the Manufacturing of Biologicals 31

Rimenys J. Carvalho and Leda R. Castilho

- 2.1 Introduction 31
- 2.2 Continuous Upstream Processes 32
 - 2.2.1 Continuous Bioprocesses: With or Without Cell Recycle? 33
 - 2.2.2 Early/Scale-Down Perfusion Development 34
 - 2.2.3 Feeding and Operational Strategies in Perfusion Processes 35
 - 2.2.4 Cell Retention Devices 36
- 2.3 Continuous Downstream Processes 41
 - 2.3.1 Continuous Liquid Chromatography (CLC) 42
 - 2.3.1.1 Continuous Annular Chromatography (CAC) 42
 - 2.3.1.2 True and Simulated Moving Bed Chromatography (TMB/SMB) 43
 - 2.3.1.3 Multicolumn Countercurrent Solvent Gradient Purification (MCSGP) 45
 - 2.3.1.4 Periodic Countercurrent Chromatography (PCC) 47
 - 2.3.1.5 Continuous Countercurrent Tangential Chromatography (CCTC) 50
 - 2.3.2 Nonchromatographic Continuous Processes 51
 - 2.3.2.1 Continuous Aqueous Two-Phase Systems 51
 - 2.3.2.2 Continuous Protein Precipitation 52
 - 2.3.3 Straight-Through Processes 53
 - 2.3.4 Continuous Virus Clearance Processes 54
- 2.4 Integrated Continuous Processes 55
- 2.5 Concluding Remarks 59
- References 60

3 Engineering Challenges of Continuous Biomanufacturing Processes (CBP) 69

Holger Thiess, Steffen Zobel-Roos, Petra Gronemeyer, Reinhard Ditz, and Jochen Strube

- 3.1 Introduction 69
 - 3.1.1 Continuous Manufacturing 69
 - 3.1.2 Continuous Manufacturing of Synthetic Molecules 69
 - 3.1.3 Continuous Manufacturing of Biologics 69
- 3.2 Analysis of CBP Status 71

3.3	Case Studies	74
3.4	Status and Needs for Research and Development	77
3.5	Engineering Challenges	79
3.5.1	Platform Method of QbD-Driven Process Modeling Instead of Unit Operation Oriented Platform Approaches	80
3.5.2	Data Driven Decisions	81
3.5.3	Analytics	82
3.5.4	QbD Methods	82
3.5.5	Upstream and Downstream Integration	82
3.5.6	Buffer Handling/Recycling	83
3.5.7	Process Integration of Innovative Unit Operations	84
3.5.8	ABC (Anything But or Beyond Chromatography) and AAC (Anything and Chromatography)	84
3.5.8.1	Liquid–Liquid Extraction Based on ATPE	84
3.5.8.2	Precipitation	86
3.5.8.3	Membrane Adsorbers	87
3.5.8.4	Innovative Materials Like Fibers or Matrices	88
3.5.9	Process Concepts for mAbs and Fragments	88
3.5.10	Single-Use Technology	91
3.5.11	Guided Decision for CBP	91
3.6	Conclusion and Outlook	96
	Acknowledgments	97
	References	97

Part Two: Automation and Monitoring (PAT) 107

4	Progress Toward Automated Single-Use Continuous Monoclonal Antibody Manufacturing via the Protein Refinery Operations Lab	109
	<i>David Pollard, Mark Brower, and Douglas Richardson</i>	
4.1	Introduction	109
4.2	Protein Refinery Operations Lab	111
4.2.1	Introduction	111
4.2.2	Protein Refinery Operations Lab: Design and Implementation	112
4.2.3	Protein Refinery Operations Lab: Process Analytical Technology (PAT) and Product Attribute Control (PAC) for the Transition to Real-Time Release (RTR)	117
4.2.3.1	Protein Refinery Operations Lab: Current State of PAT Technologies	118
4.3	Protein Refinery Operations Lab: Case Studies	122
4.3.1	Case Study: Perfusion	122
4.3.2	Case Study: Continuous Purification	124
4.3.3	Case Study: Proof of Concept Automated Handling of Deliberate Process Deviations	127
4.3.3.1	Perfusion Process Deviation Analysis (Bioreactor Temperature Shift)	127

- 4.3.3.2 Downstream Process Deviation Analysis
(Viral Inactivation pH) 128
- 4.4 Summary 129
- Acknowledgments 129
- References 129

Part Three: Single Use Technologies and Perfusion Technologies 131

- 5 Single-Use Bioreactors for Continuous Bioprocessing: Challenges and Outlook 133**
Nico M.G. Oosterhuis
 - 5.1 Introduction 133
 - 5.2 Single-Use Reactor Types 135
 - 5.3 Material Aspects 136
 - 5.4 Sensors 139
 - 5.5 Reactor Design 141
 - 5.5.1 Mass Transfer and Mixing Requirements for Continuous Processing 141
 - 5.6 Scale-Up Aspects 142
 - 5.7 Continuous Seed Train 145
 - 5.8 New Mixer Designs 145
 - 5.9 Future Outlook 146
 - References 147

- 6 Two Mutually Enabling Trends: Continuous Bioprocessing and Single-Use Technologies 149**
Marc Bisschops, Mark Schofield, and Julie Grace
 - 6.1 Introduction 149
 - 6.2 Single-Use Technologies 150
 - 6.2.1 History of Single-Use Technologies 150
 - 6.2.2 Single-Use Upstream Processing 151
 - 6.2.3 Single-Use Downstream Processing 151
 - 6.2.3.1 Tangential Flow Filtration 151
 - 6.2.3.2 Chromatography Steps 152
 - 6.2.4 Early Skepticism 152
 - 6.2.5 Current Trends and Future Predictions 153
 - 6.3 Continuous Bioprocessing 154
 - 6.3.1 Continuous Upstream Processing 154
 - 6.3.2 Continuous Downstream Processing 155
 - 6.3.2.1 Tangential Flow Filtration 156
 - 6.3.2.2 Continuous Chromatography 157
 - 6.3.3 Concerns for Continuous Bioprocessing 158
 - 6.4 Integrated Single-Use Continuous Bioprocessing: Case Studies 159
 - 6.4.1 Case 1: Genzyme 159
 - 6.4.2 Case 2: Merck 160

6.4.3	Case 3: Bayer Technology Services	161
6.4.4	Comparison	162
6.4.5	Challenges and Solutions	163
6.4.6	Alternative Scenarios	164
6.5	Regulatory Aspects	164
6.6	Adoption Rate of Single-Use and Continuous Bioprocessing	165
6.7	Conclusions	166
	References	167
7	Perfusion Formats and Their Specific Medium Requirements	171
	<i>Jochen B. Sieck, Christian Schild, and Jörg von Hagen</i>	
7.1	Introduction	171
7.1.1	History of Perfusion	172
7.1.2	Comeback of Perfusion	172
7.2	Characterization of Perfusion Processes	173
7.2.1	Productivity of Perfusion Processes	175
7.2.2	Cell Retention Devices	176
7.2.3	Steady-State Definition	176
7.3	Perfusion Formats	177
7.3.1	Innovative Perfusion Formats	178
7.4	Development Strategies for Perfusion Media	179
7.4.1	Cell Line-Specific Requirements	181
7.4.2	Scale-Down Models for Perfusion Processes	181
7.4.3	Scale-Down Cultivation Methods	182
7.4.4	Examples for Perfusion Scale-Down Applications	184
7.5	Process Development for Perfusion Processes	185
7.6	Case Study	185
7.6.1	Material & Methods	186
7.6.1.1	Semicontinuous Chemostat (SCC)	187
7.6.1.2	Repeated Batch (RB)	187
7.6.1.3	Semicontinuous Perfusion (SCP)	187
7.6.2	Results	187
7.6.2.1	Determination of the Starting Cell Density	187
7.6.3	Scale-Down Model Comparison	188
7.6.4	Media Screening	189
7.6.5	Bioreactor Confirmation	191
7.7	Conclusion	192
	Abbreviations	193
	References	194
	Part Four: Continuous Upstream Bioprocessing	201
8	Upstream Continuous Process Development	203
	<i>Sanjeev K. Gupta</i>	
8.1	Introduction	203
8.2	Upstream Processes in Biomanufacturing	205

8.2.1	Upstream Operating Modes	206
8.2.1.1	Fed-Batch Process	206
8.2.1.2	Continuous/Perfusion Process	207
8.3	The Upstream Continuous/Perfusion Process	207
8.3.1	Upstream Process-Type Selection	209
8.3.2	Component of Continuous Upstream and Downstream Processes	209
8.3.2.1	Upstream Components: Stainless Steel and Single-Use (Su)	209
8.3.2.2	Downstream Components: Stainless Steel and Single-Use (Su)	209
8.3.3	Cell Retention Devices Used in Perfusion Process	210
8.3.3.1	Spin Filters	210
8.3.3.2	The ATF System	210
8.3.3.3	Biosep Acoustic Perfusion System	212
8.3.3.4	TFF Cell Retention Device	213
8.4	Manufacturing Scale-Up Challenges	214
8.4.1	Process Complexity and Control	214
8.4.2	Cell Line Stability	215
8.4.3	Validation	215
8.5	Single-Use Technologies: A Paradigm Change	215
8.5.1	Application of SUBs in Continuous Processing	218
8.5.2	Single-Use Continuous Bioproduction	218
8.5.3	Single-Use Perfusion Bioreactors	219
8.5.3.1	Type of Single-Use Bioreactors for Perfusion Culture	219
8.5.4	Single-Use Accessories Supporting Perfusion Culture	220
8.5.4.1	Hollow Fiber Media Exchange	220
8.5.4.2	Continuous Flow Centrifugation	220
8.5.4.3	Acoustic Wave Separation	220
8.5.4.4	Spin filters	220
8.6	FDA Supports Continuous Processing	221
8.7	Making the Switch from Batch/Fed-Batch to Continuous Processing	222
8.8	Costs and Benefits of Continuous Manufacturing	222
8.9	Costs of Adoption	223
8.10	Continuous Downstream Processing	223
8.11	Integrated Continuous Manufacturing	224
8.12	Concluding Remark	227
	Acknowledgment	228
	References	228
9	Study of Cells in the Steady-State Growth Space	233
	<i>Sten Erm, Kristo Abner, Andrus Seiman, Kaarel Adamberg, and Raivo Vilu</i>	
9.1	Introduction	233
9.1.1	On Physiological State of Cells: Steady-State Growth Space Analysis	234
9.1.2	Challenge of Comprehensive Quantitative Steady-State Growth Space Analysis (SSGSA)	236
9.1.3	Chemostat Culture – A Classical Tool for SSGSA	236

9.2	Advanced Continuous Cultivation Methods – Changestats	237
9.2.1	Accelerostat (A-stat)	237
9.2.2	Family of Changestats – A Set of Flexible Tools for Scanning Steady-State Growth Space	240
9.3	Review of the Results Obtained Using the Changestats	242
9.3.1	Acetate Overflow Metabolism in <i>E. Coli</i>	242
9.3.2	A-Stat in Study of Physiology of Yeast	243
9.3.3	Integration of A-Stat with High-Throughput Omics Methods and Modeling	243
9.3.4	A-Stat in Bioprocess Development	243
9.3.5	Deceleration-stat (De-stat)	244
9.3.6	Dilution Rate Stat (D-Stat)	244
9.3.7	Auxoaccelerostats	245
9.3.8	Adaptastat	246
9.4	SSGSA Using Parallel-Sequential Cultivations	247
9.5	Modeling in Steady-State Growth Space Analysis	248
	References	250

Part Five: Continuous Downstream Bioprocessing 259

10	Continuous Downstream Processing for Production of Biotech Therapeutics	261
	<i>Anurag S. Rathore, Nikhil Kateja, and Harshit Agarwal</i>	
10.1	Introduction	261
10.2	Continuous Manufacturing Technologies for Downstream Processing	262
10.2.1	Continuous Cell Lysis	262
10.2.2	Continuous Centrifugation	263
10.2.3	Continuous Refolding	264
10.2.4	Continuous Precipitation	267
10.2.5	Continuous Chromatography	267
10.2.6	Continuous Extraction	271
10.2.7	Continuous Filtration	272
10.3	Continuous Process Development	274
10.4	Case Studies Related to Continuous Manufacturing	276
10.5	Summary	279
	References	279
11	Evolving Needs For Viral Safety Strategies in Continuous Monoclonal Antibody Bioproduction	289
	<i>Andrew Clutterbuck, Michael A. Cunningham, Cedric Geyer, Paul Genest, Mathilde Bourguignat, and Helge Berg</i>	
11.1	Introduction	289
11.1.1	Current Regulations and Practices	293
11.1.2	Evolving Needs: Process versus Regulatory	294
11.1.3	Current Technology Landscape	295
11.2	Batch versus Continuous: Potential Impacts on Virus Safety	297

- 11.2.1 Raw Material Safety/Testing 299
- 11.2.2 Upstream and Bioreactor Safety 301
- 11.2.3 Downstream Virus Removal Strategies 304
 - 11.2.3.1 Viral Reduction by Normal Flow Filtration (NFF) 304
 - 11.2.3.2 Chemical Inactivation (Low pH or Solvent Detergent) 308
 - 11.2.3.3 Chromatography 311
 - 11.2.3.4 Other Techniques 312
- 11.3 Validation of Viral Reduction Steps in Continuous Manufacturing Processes 313
 - 11.3.1 Protein A Capture Chromatography 314
 - 11.3.2 Chemical Inactivation (Low pH/Solvent Detergent) 315
 - 11.3.3 Intermediate and Polishing Chromatography 315
 - 11.3.4 Viral Reduction Filtration 316
- 11.4 Conclusion 318
- References 319

Part Six: Continuous Chromatography 321

- 12 Multicolumn Continuous Chromatography: Understanding this Enabling Technology 323**
Kathleen Mihlbachler
 - 12.1 Introduction 323
 - 12.2 Modes of Chromatography 326
 - 12.3 Interaction Mechanisms Used in Chromatographic Systems 328
 - 12.4 Batch Chromatography 330
 - 12.5 Semicontinuous and Continuous Batch Chromatography 331
 - 12.5.1 Single Column 331
 - 12.5.2 Multicolumn Parallel Operation 333
 - 12.5.3 Multicolumn Parallel and Interconnected Operation 337
 - 12.6 Multicolumn, Countercurrent, Continuous Chromatography 340
 - 12.6.1 Implementing Traditional SMB Technology 341
 - 12.6.2 SMB Technology for Biomolecules 343
 - 12.6.3 Additional Examples of SMB Purifications 349
 - 12.7 Risk Assessment of Continuous Chromatography 353
 - 12.8 Process Design of Continuous Capture Step 357
 - 12.9 Conclusion 360
 - References 361

- 13 Continuous Chromatography as a Fully Integrated Process in Continuous Biomanufacturing 369**
Steffen Zobel-Roos, Holger Thiess, Petra Gronemeyer, Reinhard Ditz, and Jochen Strube
 - 13.1 Introduction 369
 - 13.2 Continuous Chromatography 370
 - 13.2.1 SMB 370
 - 13.2.2 Serial Multicolumn Continuous Chromatography 377

- 13.2.3 Continuous Countercurrent Multicolumn Gradient Chromatography 378
- 13.2.4 Integrated Countercurrent Chromatography 379
- 13.3 Conclusion and Outlook 386
 - Symbols 388
 - References 389

- 14 Continuous Chromatography in Biomanufacturing 393**
Thomas Müller-Späth and Massimo Morbidelli
- 14.1 Introduction to Continuous Chromatography 393
- 14.2 Introduction to Manufacturing Aspects of Chromatography 396
- 14.3 Trade-Offs in Batch Chromatography 399
- 14.4 Capture Applications 400
 - 14.4.1 Introduction 400
 - 14.4.2 Process Principle 403
 - 14.4.3 Application Examples 405
- 14.5 Polishing Applications 406
 - 14.5.1 Introduction 406
 - 14.5.2 MCSGP (Multicolumn Countercurrent Solvent Gradient Purification) Principle 407
 - 14.5.3 MCSGP (Multicolumn Countercurrent Solvent Gradient Purification) Process Design 409
 - 14.5.4 MCSGP (Multicolumn Countercurrent Solvent Gradient Purification) Case Study 412
- 14.6 Discovery and Development applications 414
- 14.7 Scale-Up of Multicolumn Countercurrent Chromatography Processes 416
- 14.8 Multicolumn Countercurrent Chromatography as Replacement for Batch Chromatography Unit Operations 417
- 14.9 Multicolumn Countercurrent Chromatography and Continuous Upstream 419
- 14.10 Regulatory Aspects and Control of Multicolumn Countercurrent Processes 419
 - References 421

- 15 Single-Pass Tangential Flow Filtration (SPTFF) in Continuous Biomanufacturing 423**
Andrew Clutterbuck, Paul Beckett, Renato Lorenzi, Frederic Sengler, Torsten Bisschop, and Josselyn Haas
- 15.1 Introduction 423
- 15.2 Tangential Flow Filtration in Bioproduction 426
 - 15.2.1 Batch versus Single-Pass Tangential Flow Filtration 426
 - 15.2.2 Membrane Type and Format for TFF Applications 426
 - 15.2.3 Single-Pass Tangential Flow Filtration (SPTFF) 428
 - 15.2.4 Process Design 430
 - 15.2.5 Laboratory-Scale Process Development Example 438

- 15.2.6 Consideration on Equipment Configuration and Requirements 442
- 15.3 Validation 445
- 15.3.1 Key Validation Considerations between Batch and Continuous Processing 445
- 15.3.2 Validation of Single-Pass TFF 449
- 15.4 Conclusion 453
- References 453

Part Seven: Integration of Upstream and Downstream 457

16 Design of Integrated Continuous Processes for High-Quality Biotherapeutics 459

Fabian Steinebach, Daniel Karst, and Massimo Morbidelli

- 16.1 Introduction 459
- 16.2 Perfusion Cell Culture Development 463
- 16.2.1 Objectives and Requirements 463
- 16.2.2 Bioreactor Setup 463
- 16.2.3 Physical Bioreactor Characterization 464
- 16.3 Continuous Capture Development 466
- 16.3.1 Objectives and Requirements 466
- 16.3.2 Continuous Two-Column Capture Process 467
- 16.3.3 Process Performance 468
- 16.3.4 Process Control 469
- 16.4 Operation of the Continuous Integrated Process 470
- 16.4.1 Bioreactor Operation 470
- 16.4.2 Cell Growth 470
- 16.4.3 Monoclonal Antibody Production 471
- 16.4.4 Monoclonal Antibody Capture 472
- 16.4.5 Process Performance 473
- 16.4.6 Product Quality 474
- 16.5 Conclusion 476
- Acknowledgment 477
- References 477

17 Integration of Upstream and Downstream in Continuous Biomanufacturing 481

Petra Gronemeyer, Holger Thiess, Steffen Zobel-Roos, Reinhard Ditz, and Jochen Strube

- 17.1 Introduction 481
- 17.2 Background on Upstream Development in Continuous Manufacturing 483
- 17.3 Background on Downstream Development in Continuous mAb Manufacturing 484
- 17.4 Challenges in Process Development 485
- 17.4.1 Impact of Changing Titers and Impurities on Cost Structures 485

- 17.4.2 Impurities as Critical Parameters in Process Development 487
- 17.4.3 Host Cell Proteins as Main Problem in Process Development 488
- 17.4.4 Regulatory Aspects 490
- 17.5 Trends and Integration Approaches 490
- 17.6 Methodical Approach of Integrating USP and DSP Regarding Impurity Processing 492
 - 17.6.1 Case Study: Influence of Media Components on Impurity Production 494
 - 17.6.2 Case Study: Influence of Harvest Operations on Impurity Production 495
 - 17.6.3 Nonchromatographic Continuous DSP Operation 497
 - 17.6.3.1 ATPS 498
 - 17.6.3.2 Precipitation 499
 - 17.6.3.3 One Step Toward a Chromatography Free Purification Process 500
- 17.7 Conclusion and Outlook 500
 - References 501

Part Eight: Quality, Validation, and Regulatory Aspects 511

- 18 Quality Control and Regulatory Aspects for Continuous Biomanufacturing 513**
Guillermina Forno and Eduardo Ortí
 - 18.1 Introduction 513
 - 18.2 FDA Support for Continuous Manufacturing 513
 - 18.3 PAT as a Facilitator for Continuous Manufacturing Implementation 514
 - 18.4 PAT Applications in the Pharmaceutical Industry 516
 - 18.5 Process Validation for Continuous Manufacturing Processes 519
 - 18.6 Regulatory Documents Related to Process Validation 520
 - 18.7 ICH 520
 - 18.8 FDA 520
 - 18.9 EMA 521
 - 18.10 ASTM 521
 - 18.11 Special Considerations for Continuous Manufacturing Process Validation 521
 - 18.12 Scale-Down for Continuous Bioprocessing 524
 - 18.13 Impact of Single-Use Systems in Process Validation 526
 - 18.14 Batch and Lot Definition 527
 - 18.15 Conclusion 528
 - References 528
- 19 Continuous Validation For Continuous Processing 533**
Steven S. Kuwahara
 - 19.1 Quality Management 533

19.2	Regulatory Considerations	534
19.3	Setting Specifications	534
19.4	Sequence of Events	535
19.5	Verification of Validated States	536
19.6	Choice of Test Methods	536
19.7	Types of Monitoring	536
19.8	Process Stream Analyzers	538
19.9	Validation/Qualification of Process Stream Analyzers	538
19.10	Control Charting	540
19.11	The Moving Range Chart	541
19.12	Continuous Validation	541
19.13	Choosing Other Control Charts	542
19.14	Information Awareness	542
19.15	Cost Issues	543
19.16	Revalidations	544
19.17	Management and Personnel	544
	References	545

20 Validation, Quality, and Regulatory Considerations in Continuous Biomanufacturing 549

Laura Okhio-Seaman

20.1	Introduction	549
20.1.1	What is Continuous Biomanufacturing?	549
20.1.2	Improvement in Product Quality	550
20.1.3	Manufacturing Consistency	550
20.1.4	Efficient Facility and Personnel Utilization	550
20.1.5	Reduction in Capital Expenditure and Cost	550
20.2	Quality	551
20.2.1	Other Considerations in Quality	552
20.2.1.1	Contract Manufacturing Organizations (CMO's)	552
20.2.1.2	Good Manufacturing Practices (GMP)	555
20.2.1.3	Supply Chains	555
20.2.1.4	Change Management and Control	556
20.3	Validation	557
20.3.1	Validate to Eliminate!	557
20.3.2	Test Conditions for Extractable and Leachable Analysis	560
20.3.3	Test Solutions for Extractable and Leachable Analysis	561
20.3.4	Analytical Techniques for Leachables Analysis	561
20.3.5	Description of the Model Approach	562
20.3.6	Actual Formulation Approach	563
20.4	Regulatory	564
20.4.1	Current Regulatory References	565
20.5	Conclusion	566
	Further Reading	566

Part Nine: Industry Perspectives 569

- 21 Evaluation of Continuous Downstream Processing: Industrial Perspective 571**
Venkatesh Natarajan, John Pieracci, and Sanchayita Ghose
- 21.1 Biogen mAb Downstream Platform Process 571
- 21.2 Potential Platform Process Bottlenecks Pertaining to Large Scale Manufacturing 573
- 21.3 Continuous Downstream Process 573
 - 21.3.1 Multicolumn Chromatography (MCC) for Continuous Capture 575
 - 21.3.1.1 Background 575
 - 21.3.1.2 Process Optimization 576
 - 21.3.1.3 Experimental Results 577
 - 21.3.2 Continuous Viral Inactivation 578
 - 21.3.3 Connected Chromatography Steps 580
 - 21.3.3.1 Comparison of Current and Pool-Less Process 581
 - 21.3.4 Continuous UF/DF Processes 582
- 21.4 Productivity Comparison of Batch and Continuous Downstream Process 585
 - References 585

- Index 587**

List of Contributors

Kristo Abner

Competence Center of Food and
Fermentation Technologies
Akadeemia tee 15
12618 Tallinn
Estonia

Kaarel Adamberg

Tallinn University of Technology
Department of Chemistry and
Biotechnology
Akadeemia tee 15
12618 Tallinn
Estonia

and

Competence Center of Food and
Fermentation Technologies
Akadeemia tee 15
12618 Tallinn
Estonia

Harshit Agarwal

Indian Institute of Technology
Department of Chemical
Engineering
Hauz Khas
110016 New Delhi
India

Paul Beckett

Millipore SAS
Process Solution Technologies
39 Route Industrielle de la Hardt
67124 Molsheim
France

Helge Berg

Technology Management
Millipore SAS
39 Route Industrielle de la Hardt
67124 Molsheim
France

Torsten Bisschop

Millipore SAS
Process Solution Technologies
39 Route Industrielle de la Hardt
67124 Molsheim
France

Marc Bisschops

Pall Life Sciences
Scientific Laboratory Services
Nijverheidsweg 1
1671 GC Medemblik
The Netherlands

Mathilde Bourguignat

Technology Management
Millipore SAS
39 Route Industrielle de la Hardt
67124 Molsheim
France

Mark Brower

Merck & Co Inc
Biologics & Vaccines
2000 Galloping Hill Road
Kenilworth, NJ 07033
USA

Rimenys J. Carvalho

Federal University of Rio de Janeiro
COPPE
Cell Culture Engineering Laboratory
C.P. 68502
21941-972 Rio de Janeiro, RJ
Brazil

Leda R. Castilho

Federal University of Rio de Janeiro
COPPE
Cell Culture Engineering Laboratory
C.P. 68502
21941-972 Rio de Janeiro, RJ
Brazil

Cedric Geyer

Technology Management
Millipore SAS
39 Route Industrielle de la Hardt
67124 Molsheim
France

Andrew Clutterbuck

Millipore SAS
Process Solution Technologies
39 Route Industrielle de la Hardt
67124 Molsheim
France

M. Nicolas Cruz-Bournazou

Technische Universität Berlin
Department of Biotechnology
Ackerstrasse 76
ACK 24
13355 Berlin
Germany

Michael A. Cunningham

Technology Management
EMD Millipore Corporation
290 Concord Road
Billerica, MA 01821
USA

Reinhard Ditz

Clausthal University of Technology
Institute for Separation and Process
Technology
Leibnizstr 15
38678 Clausthal-Zellerfeld
Germany

Sten Erm

Tallinn University of Technology
Department of Chemistry and
Biotechnology
Akadeemia tee 15
12618 Tallinn
Estonia

and

Competence Center of Food and
Fermentation Technologies
Akadeemia tee 15
12618 Tallinn
Estonia

Guillermina Forno

Ciudad Universitaria
Cell Culture Laboratory
UNL
FBCB
Paraje el Pozo
CC 242 Santa Fe
Argentina

and

Ciudad Universitaria
R&D Zelltek S.A.
UNL
FBCB
Paraje el Pozo
CC 242 Santa Fe
Argentina

Paul Genest

Technology Management
EMD Millipore Corporation
290 Concord Road
Billerica, MA 01821
USA

Sanchayita Ghose

Bristol-Myers Squibb
Downstream Process Development
38 Jackson Road
Danvers, MA 01923
USA

Julie Grace

Pall Life Sciences
Scientific Laboratory Services
20 Walkup Drive
Westborough, MA 01581
USA

Petra Gronemeyer

Clausthal University of Technology
Institute for Separation and Process
Technology
Leibnizstr 15
38678 Clausthal-Zellerfeld
Germany

Sanjeev K. Gupta

Ipsca Laboratories Ltd.
Advanced Biotech Lab
Kandivli Industrial Estate
Kandivli (west)
400067 Mumbai
India

Josselyn Haas

Millipore SAS
Process Solution Technologies
39 Route Industrielle de la Hardt
67124 Molsheim
France

Daniel Karst

ETH Zurich
Institute for Chemical and
Bioengineering
Department of Chemistry and
Applied Biosciences
Vladimir-Prelog-Weg 1
8093 Zurich
Switzerland

Nikhil Kateja

Indian Institute of Technology
Department of Chemical
Engineering
Hauz Khas
110016 New Delhi
India

Steven S. Kuwahara

GXP BioTechnology LLC
Tucson, AZ 85741
USA

Renato Lorenzi

Millipore SAS
Process Solution Technologies
39 Route Industrielle de la Hardt
67124 Molsheim
France

Kathleen Mihlbachler

Lewa Process Technologies
Inc. Separations Development
8 Charlestown Street
Devens, MA 01434
USA

Massimo Morbidelli

ETH Zurich
Institute for Chemical and
Bioengineering
Department of Chemistry and
Applied Biosciences
Vladimir-Prelog-Weg 1
8093 Zürich
Switzerland

Thomas Müller-Späth

ChromaCon AG
Process Development
Technoparkstrasse 1
8005 Zurich
Switzerland

and

ETH Zurich
Institute for Chemical and
Bioengineering
Department of Chemistry and
Applied Biosciences
Vladimir-Prelog-Weg 1
8093 Zürich
Switzerland

Venkatesh Natarajan

Biogen
Engineering & Technology
225 Binney Street
Cambridge, MA 02142
USA

Peter Neubauer

Technische Universität Berlin
Department of Biotechnology
Ackerstrasse 76
ACK 24
13355 Berlin
Germany

Laura Okhio-Seaman

Sartorius Stedim North America
Validation Services
5 Orville Drive
Bohemia, NY 11716
USA

Nico M.G. Oosterhuis

Celltainer Biotech BV
Bothoekweg 9
7115AK Winterswijk
The Netherlands

Eduardo Ortí

Ciudad Universitaria
R&D Zelltek S.A.
UNL
FBCB
Paraje el Pozo
CC 242 Santa Fe
Argentina

John Pieracci

Biogen
Engineering & Technology
225 Binney Street
Cambridge, MA 02142
USA

David Pollard

Merck & Co Inc
Biologics & Vaccines
2000 Galloping Hill Road
Kenilworth, NJ 07033
USA

Anurag S.Rathore

Indian Institute of Technology
Department of Chemical
Engineering
Hauz Khas
110016 New Delhi
India

Douglas Richardson

Merck & Co Inc
 Biologics & Vaccines
 2000 Galloping Hill Road
 Kenilworth, NJ 07033
 USA

Christian Schild

Merck Life Science
 (a business of Merck KGaA) Process
 Solutions
 Cell Culture Media R&D
 Frankfurter Strasse 250
 64291 Darmstadt
 Germany

Mark Schofield

Pall Life Sciences
 Applications R&D
 20 Walkup Drive
 Westborough, MA 01581
 USA

Andrus Seiman

Tallinn University of Technology
 Department of Chemistry and
 Biotechnology
 Akadeemia tee 15
 12618 Tallinn
 Estonia

and

Competence Center of Food and
 Fermentation Technologies
 Akadeemia tee 15
 12618 Tallinn
 Estonia

Frederic Sengler

Millipore SAS
 Process Solution Technologies
 39 Route Industrielle de la Hardt
 67124 Molsheim
 France

Jochen B. Sieck

Merck Life Science
 (a business of Merck KGaA)
 Process Solutions
 Cell Culture Media R&D
 Frankfurter Strasse 250
 64291 Darmstadt
 Germany

Fabian Steinebach

ETH Zurich
 Institute for Chemical and
 Bioengineering
 Department of Chemistry and
 Applied Biosciences
 Vladimir-Prelog-Weg 1
 8093 Zurich
 Switzerland

Jochen Strube

Clausthal University of Technology
 Institute for Separation and Process
 Technology
 Leibnizstr 15
 38678 Clausthal-Zellerfeld
 Germany

Holger Thiess

Clausthal University of Technology
 Institute for Separation and Process
 Technology
 Leibnizstr 15
 38678 Clausthal-Zellerfeld
 Germany

Raivo Vilu

Tallinn University of Technology
 Department of Chemistry and
 Biotechnology
 Akadeemia tee 15
 12618 Tallinn
 Estonia

and

Competence Center of Food and
Fermentation Technologies,
Akadeemia tee 15
12618 Tallinn
Estonia

Jörg von Hagen

Merck Life Science (a business of
Merck KGaA) Process Solutions
Cell Culture Media R&D
Frankfurter Strasse 250
64291 Darmstadt
Germany

Steffen Zobel-Roos

Clausthal University of Technology
Institute for Separation and Process
Technology
Leibnizstr 15
38678 Clausthal-Zellerfeld
Germany

Part One

Overview of State-of-the-Art Technologies and Challenges

1

Continuous Bioprocess Development: Methods for Control and Characterization of the Biological System

Peter Neubauer and M. Nicolas Cruz-Bournazou

Technische Universität Berlin, Department of Biotechnology, Ackerstrasse 76, ACK 24, 13355 Berlin, Germany

1.1 Proposed Advantages of Continuous Bioprocessing

1.1.1 Introduction

The change from batch to continuous processing has led to the intensification of processes in a number of industries, including steel casting, automobile and other devices, petrochemicals, food, and pharmaceuticals. Advantages include, aside from a significant increase in volumetric productivity, reduced equipment size, steady-state operation, low cycle times, streamed process flows, and reduced capital cost.

In bioengineering, continuous processing is the standard in wastewater treatment, composting, and some bioenergy processes such as biogas and bioethanol fermentations. In contrast, most production processes run as batch type operations or more specifically fed-batch processes, which is the major production technology today.

Konstantinov and Cooney provide a definition of a continuous process as “A unit operation is continuous if it is capable of processing a continuous flow input for prolonged periods of time. A continuous unit operation has minimal internal hold volume. The output can be continuous or discretized in small packets produced in a cyclic manner.” [1]. They also differentiate between full continuous processes with no or minimal hold volume in the process line or hybrid processes that contain both batch and continuous process operations.

Obviously, the push in continuous manufacturing technologies was initiated by the BioPAT initiative of the Food and Drug Administration (FDA) in 2002 and the published guidance to PAT in 2004 [2], which initially aimed at a better understanding of the connections between product quality and process conditions. This led to the need to develop quality by design (QbD), that is, the implementation of process analytical tools over the whole developmental pipeline from early product screening over the process development in the laboratory scale and during scale up. The needs for a better understanding of the impact of process parameters on the critical quality attributes (CQA) of the respective product also increased the interest in the development and implementation of novel sensors and analytical

tools. As a consequence, this better understanding of processes resulted in further process intensification and provided the instrumental basis to approach challenges in relation to continuous operation.

Aside the FDA initiative, there are several drivers for the increasing interest in continuous processing, not only in the pharmaceutical industry but also in the industrial (white) biotech industry. On one side, we see an increasing demand and thus also increasing production scale for industrial bioproducts (enzymes, small molecules, and bioenergy market) with a need for reduced costs for the products and increased competition. Considering that production scales are steadily growing and that a scale reduction close to factor 10 would be possible by continuous processing, plant sizes and the efficiency of bioprocesses could be increased significantly. On the other side, the opportunity of the selection of new biocatalysts and its implementation in the chemical synthesis for integrated chemoenzymatic processes (i.e., processes which combine chemical and enzymatic reactions) have to be competitive with the existing chemical processes and need to be integrated into the chemical production schemes. Here, continuous processes offer clear advantages.

In biopharma for recombinant proteins, antibodies, highly complex proteins, recombinant enzymes and blood factors, the efficiency of the cell factories, and production systems have dramatically increased during the last decade. Opportunities for high cell density processes with a higher volumetric product yield and quality, as well as the changing situation in view of the intellectual properties by the termination of many patents for important drugs with novel commercial opportunities for new biosimilars and biobetters are a strong driver in increasing the competition especially from emerging markets. In parallel, there is an increasing demand for establishing local production sites for defined regional markets, rather than having single production sites. Strict cost calculations as a developmental driver demand for smaller and effective, but also flexible production plants. This directs interest to evaluate continuous bioprocessing opportunities to minimize investments for production facilities, and thinking about parallelization rather than larger scales. Parallelization would also be an advantage in processes with longer plant cycle times [3] as, for example, cell culture-based products. A nice example that shows the opportunities in significantly decreasing operational and capital expenses by changing from conventional bioprocessing to continuous bioprocessing in the case of production on monoclonal antibodies (mAB) and other non-mAB processes is shown by Walther *et al.* [4].

However, despite the obvious opportunities of continuous processes there are many challenges to solve, mainly the demand for fast realization and risk minimization. Currently, it seems to be easier to transfer a batch process into production than to start a new, longer, and more expensive development of a continuous process even though it is expected to be more efficient.

These scenarios show that there is a big need in strategic methods concerning the development of continuous process strategies for either new products or to derive a continuous process from existing batch type processes. As early-phase product development can practically be only performed as batch processes, a key question in product development is how we can transfer a batch strategy to a continuous operation in a large process.