

Table of content

Vorwort und Danksagung	V
Abstract.....	VII
Zusammenfassung.....	IX
Table of content.....	XI
Abbreviations and symbols	XV
1 Motivation and background	1
1.1 Industrial biotechnology	2
1.2 Bioprocess development.....	2
1.2.1 Reactor inhomogeneity	2
1.2.2 Population heterogeneity.....	3
1.3 Microfluidic single-cell analysis	4
1.4 Project objectives	5
1.5 Workflow	6
1.6 Thesis outline	7
2 Technology overview	11
2.1 Microbial single-cell analysis	12
2.1.1 Abstract	12
2.1.2 Introduction	12
2.1.3 Single-cell analysis: snapshot vs. dynamic	13
2.1.4 Microfluidic single-cell cultivation - Physics and fabrication	16
2.1.5 Microfluidic single-cell cultivation systems - Overview and examples	19
2.1.6 Challenges and future directions	32
2.1.7 Conclusions	33
2.2 Single-cell microfluidics for bioprocess development	34
2.2.1 Abstract	34
2.2.2 Introduction	34
2.2.3 Application fields.....	38
2.2.4 Conclusion	43
3 Single-cell microfluidics: Development and validation	45
3.1 Microfabrication	46
3.1.1 Abstract	46
3.1.2 Introduction	46

3.1.3	Protocol	48
3.1.4	Representative results	52
3.1.5	Discussion	58
3.2	Picoliter bioreactors	61
3.2.1	Abstract	61
3.2.2	Introduction	61
3.2.3	Device principle and design	64
3.2.4	Material and methods	66
3.2.5	Results and discussion	68
3.2.6	Conclusions	74
3.3	High-throughput single-cell cultivation	76
3.3.1	Abstract	76
3.3.2	Introduction	76
3.3.3	Materials and methods	78
3.3.4	Results	80
3.3.5	Discussion	89
3.3.6	Conclusion	91
3.4	Systematic comparison of single-cell cultivation technologies	92
3.4.1	Abstract	92
3.4.2	Introduction	93
3.4.3	Materials and methods	94
3.4.4	Results	97
3.4.5	Discussion	106
4	Single-cell analysis in bioprocess development	111
4.1	Growth rate investigation I: Cultivation at different scales	112
4.1.1	Abstract	112
4.1.2	Introduction	112
4.1.3	Material and methods	116
4.1.4	Results and discussion	119
4.1.5	Conclusions and outlook	125
4.2	Growth rate investigation II: What triggers faster growth ?	126
4.2.1	Abstract	126
4.2.2	Introduction	126
4.2.3	Materials and methods	128
4.2.4	Results and discussion	131
4.2.5	Conclusions	143
4.3	Carbon source dependent cell size and growth of <i>C. glutamicum</i>	145
4.3.1	Abstract	145

4.3.2	Introduction	145
4.3.3	Materials and methods	148
4.3.4	Results	150
4.3.5	Discussion	154
4.3.6	Conclusion	156
5	Future application	157
5.1	Optimization of single-cell designs.....	158
5.1.1	Continuous colony reactor	158
5.1.2	Single-cell growth channels	159
5.1.3	Single-cell traps.....	160
5.2	Further fields of application.....	160
5.2.1	Optimization for industrial platform organisms.....	160
5.2.2	Fluorescence coupled growth and production studies.....	164
5.2.3	Co-cultivation.....	166
6	Final conclusion	167
6.1	Summary and conclusions.....	168
6.2	Recommendations	169
6.2.1	Platform optimization and characterization	169
6.2.2	Cultivation parameter and analysis	171
6.2.3	Single-cell manipulation	172
6.2.4	Automated image analysis and visualization	172
6.2.5	Data management.....	173
Appendix		175
Appendix A		175
Appendix B		178
Appendix C		181
Appendix D		193
Appendix E		194
Appendix F		202
Appendix G		207
References		208
Publications		224