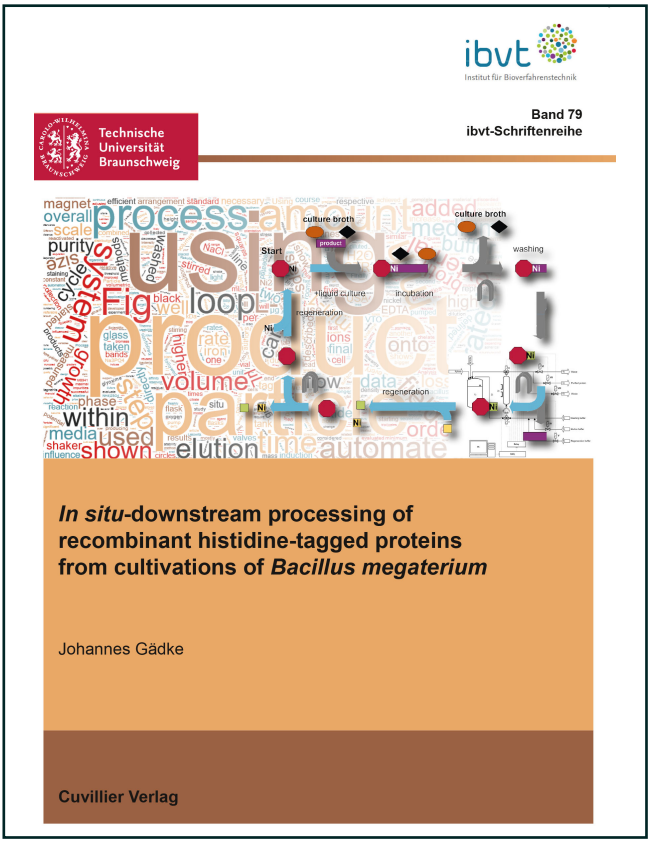
Johannes Gädke (Autor)

In situ-downstream processing of recombinant histidine-tagged proteins from cultivations of Bacillus megaterium



https://cuvillier.de/de/shop/publications/7546

Copyright:
Cuvillier Verlag, Inhaberin Annette Jentzsch-Cuvillier, Nonnenstieg 8, 37075 Göttingen, Germany
Telefon: +49 (0)551 54724-0, E-Mail: info@cuvillier.de, Website: https://cuvillier.de

Index

Indices of abbreviations and symbols I

1 Introduction and aims of the thesis 1

2 Current state of research 6

2.1 Bacterial production systems 6

2.1.1 Bacillus megaterium 7

2.1.2 Protein A 9

2.1.3 Antibodies and α-Lysozyme D1.3scFv 10

2.2 Affinity and affinity chromatography 12

2.2.1 Affinity tags 12

2.3 Superparamagnetic iron oxide nanoparticles for product purification 14

2.3.1 Functionalization using divalent metal cations 16

2.3.2 Magnetic separation of super paramagnetic iron oxide nanoparticles 17

2.3.3 Particle systems used in the course of this scientific work 18

3 Materials and methods 20

3.1 Separation and simulation 20

3.1.1 Particle systems 20

3.1.2 Separation using handheld magnets 21

3.1.3 Parameter screening for particle separation in an external loop 21

3.1.4 Simulation of magnetic field lines 22

3.2 Cultivation protocols 22

3.2.1 Strain construction 22

3.2.2 Shake flask cultivation and strain maintenance 24

3.2.3 Screening of plasmids for highest productivity 26

3.2.4 Adsorption isotherms 26

***3.2.5 In situ*-product removal from shake flask cultivations** 27

3.2.6 *In situ*-product removal from reactor cultivations (partly automated) 29

3.2.7 *In situ*-product removal from reactor cultivations (automated) 31

3.2.8 Cleaning in place of automated reactor set-up 33

3.3 Analysis protocols 33

3.3.1 Nickel leakage 33

3.3.2 Quantitative product analysis 34

Dieses Werk ist copyrightgeschützt und darf in keiner Form vervielfältigt werden noch an Dritte weitergegeben werden.
Es gilt nur für den persönlichen Gebrauch.

3.3.3 Qualitative product and quantitative purity analysis 34
3.3.4 Protein purification for production of standards 34
3.3.5 HPLC analysis of carbon sources and acetate 37

4 Results and discussion 38
4.1 Enhancing the efficiency in product analytics 38
4.2 Cultivation of recombinant *B. megaterium* and production of recombinant Protein A in shake flasks 41
4.3 Pre-experiments for *in situ*-affinity purification using recombinant Protein A 42
4.4 *In situ*-separation in shake flask cultivation 48
4.4.1 Protein A separation and product purity using GNTA-SPION 48
4.4.2 Protein A separation and product purity using SD-SPION 54
4.5 Pre-experiments for automated and integrated product separation in a lab-scale stirred tank bioreactor cultivation 59
4.5.1 Automation of particle separation 59
4.5.2 Simulation of magnetic fields and evaluation of particle separation using GNTA-SPION 61
4.5.3 Particle separation using SD-SPION 67
4.5.4 *B. megaterium* cultivation in a lab-scale stirred tank bioreactor 69
4.6 Integrated *in situ*-separation of Protein A in stirred tank bioreactor cultivation 70
4.7 Automated *in situ*-separation of Protein A in stirred tank bioreactor cultivation 72
4.8 Overall comparison of shake flask and stirred tank bioreactor cultivations using GNTA- and SD-SPION for purification of recombinant Protein A 79
4.9 Production of recombinant D1.3scFv 81
4.10 Automated *in situ*-separation of D1.3scFv antibody fragment in stirred tank bioreactor cultivation 84

5 Conclusion and outlook 87

6 Literature 90

Dieses Werk ist copyrightgeschützt und darf in keiner Form vervielfältigt werden noch an Dritte weitergegeben werden.
Es gilt nur für den persönlichen Gebrauch.