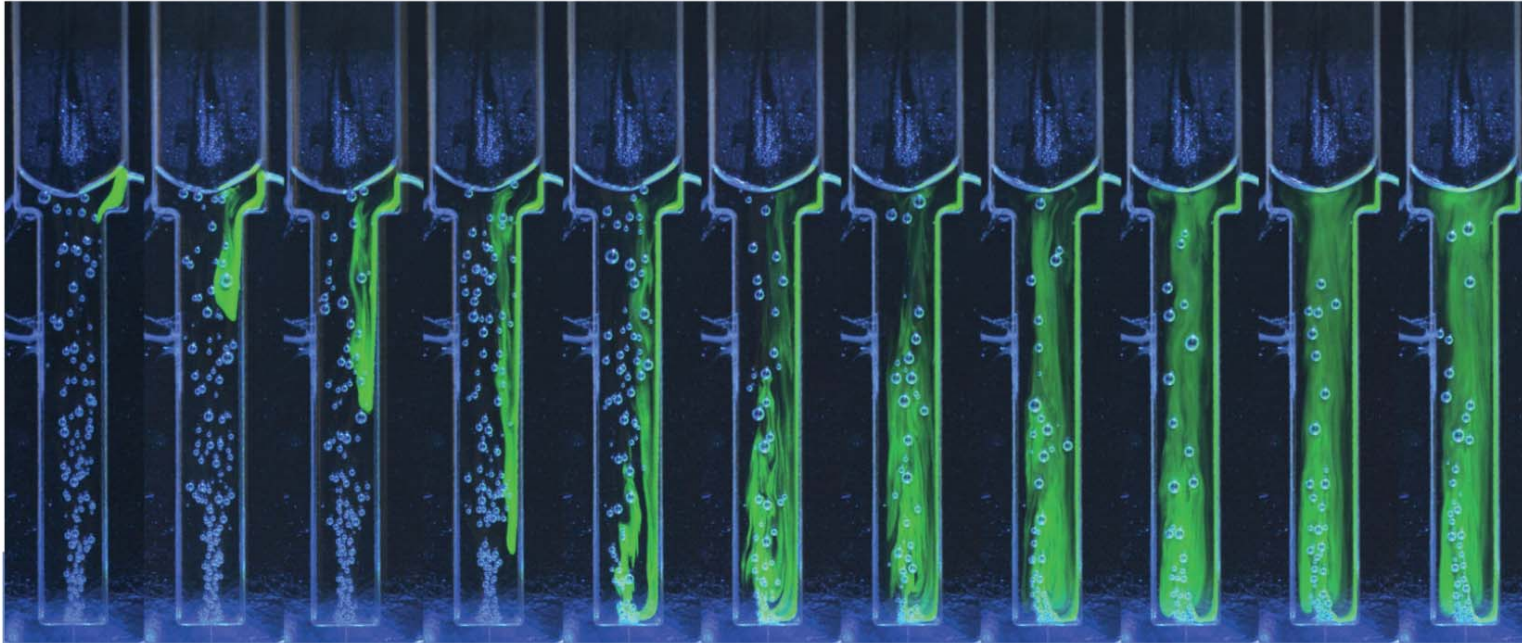




Bioengineering at the micro-scale: Design, characterization and validation of microbioreactors

Susanna Maria Lladó Maldonado



ibvt-Schriftenreihe

Schriftenreihe des Institutes für Bioverfahrenstechnik
der Technischen Universität Braunschweig

Herausgegeben von Prof. Dr. Rainer Krull

Band 81

**Cuvillier-Verlag
Göttingen, Deutschland**



Herausgeber
Prof. Dr. Rainer Krull
Institut für Bioverfahrenstechnik
TU Braunschweig
Rebenring 56, 38106 Braunschweig
www.ibvt.de

Hinweis: Obgleich alle Anstrengungen unternommen wurden, um richtige und aktuelle Angaben in diesem Werk zum Ausdruck zu bringen, übernehmen weder der Herausgeber, noch der Autor oder andere an der Arbeit beteiligten Personen eine Verantwortung für fehlerhafte Angaben oder deren Folgen. Eventuelle Berichtigungen können erst in der nächsten Auflage berücksichtigt werden.

Bibliographische Informationen der Deutschen Nationalbibliothek

Die Deutsche Nationalbibliothek verzeichnet diese Publikation in der Deutschen Nationalbibliographie; detaillierte bibliographische Daten sind im Internet über <http://dnb.d-nb.de> abrufbar.

1. Aufl. – Göttingen: Cuvillier, 2019

© Cuvillier-Verlag · Göttingen 2019
Nonnenstieg 8, 37075 Göttingen
Telefon: 0551-54724-0
Telefax: 0551-54724-21
www.cuvillier.de

Alle Rechte, auch das der Übersetzung, vorbehalten

Dieses Werk – oder Teile daraus – darf nicht vervielfältigt werden, in Datenbanken gespeichert oder in irgendeiner Form – elektronisch, fotomechanisch, auf Tonträger oder sonst wie – übertragen werden ohne die schriftliche Genehmigung des Verlages.

1. Auflage, 2019
Gedruckt auf säurefreiem Papier

ISBN 978-3-7369-7025-0
eISBN 978-3-7369-6025-1
ISSN 1431-7230



Bioengineering at the micro-scale: Design, characterization and validation of microbioreactors

Bei der Fakultät für Maschinenbau
der Technischen Universität Carolo-Wilhelmina zu Braunschweig

zur Erlangung der Würde
einer Doktor-Ingenieurin (Dr.-Ing.)
eingereichte Dissertation

von

Ing. Química, M. Sc. Susanna Maria Lladó Maldonado
aus Barcelona

eingereicht am: 07.06.2018

mündliche Prüfung am: 27.09.2018

Vorsitz: Prof. Dr. Andreas Dietzel

Gutachter: Prof. Dr. Rainer Krull

Prof. Dr. Ulrich Krühne

2019





I am among those who think that science has great beauty.

Marie Skłodowska-Curie



Acknowledgements

The present thesis was developed during my work as PhD candidate at the Institute of Biochemical Engineering of the Technische Universität Braunschweig and it was funded by the People Programme (Marie Curie Actions, Multi-ITN) of the European Union's Seventh Framework Programme for research, technological development and demonstration within the project *EUROMBR — European network for innovative microbioreactor applications in bioprocess development* (Project ID 608104). I also received an additional six-month funding from the *Promotionsabschlussförderung* program of the Gleichstellungsbüro of the TU Braunschweig.

First of all, I would like to thank my supervisor Prof. Dr. habil. Rainer Krull, my *Doctorfather*, for his passion, engagement, and all the support all the way through the project.

I would like to show my gratitude to Prof. Dr. Ulrich Krühne, Process and Systems Engineering Center (PROSYS), Department of Chemical and Biochemical Engineering, Technical University of Denmark, for his invaluable help with *CFD*, and for accepting being part of the dissertation committee.

Thanks to Prof. Dr. Andreas Dietzel from the Institute of Microtechnology, TU Braunschweig, for taking over the presidency of the dissertation committee.

A huge thank to Detlev Rasch for being extremely helpful in the laboratory set-up. Thanks to Dr.-Ing. Astrid Edlich for reviewing my work.

I would like to thank the *EUROMBR* family for all the interesting discussions and other unforgettable moments. *EUROMBR* has given me access to a great network of international experts in the field, the chance of participating into high quality trainings and conferences, and living new enriching experiences. A special thanks to the cooperation partners for the fruitful collaborations: Prof. Dr. Ulrich Krühne (Process and Systems Engineering Center (PROSYS), Department of Chemical and Biochemical Engineering, Technical University of Denmark), Prof. Dr. Torsten Mayr and Shiwen Sun (Institute of Analytical Chemistry and Food Chemistry, Graz University of Technology, Austria), Dr. Adama M. Sesay and Peter Panjan



(Measurement Technology Unit, University of Oulu, Kajaani, Finland), Dr. Juan M. Bolivar and Donya Valikhani (Institute of Biotechnology and Biochemical Engineering, Graz University of Technology, Austria) and Prof. Nicolas Szita and Dr. Marco Marques (Department of Biochemical Engineering, University College London, United Kingdom).

I am thankful to the most dedicated and motivated students Jana Krull (in cooperation with the Microfluidics Laboratory in the Department of Biochemical Engineering, University College London, United Kingdom) and Leopold Heydorn (in cooperation with the Institute of Biotechnology and Biochemical Engineering, Graz University of Technology, Austria) for pursuing their master thesis in the field of microbioeactors.

My greatest gratitude to the *ibvt* colleagues for the pleasant working atmosphere, and specially my officemates Christina, Lasse, and Leopold who would listen and help in any moment.

I would also thank to my flatmates from the Bültenweg WG, for being my family away from home. I would especially thank my dearest friend Elena, for taking care of me, being always in the positive side, and laughing with me.

To my family and friends in Spain, that despite the distance I could feel them very close to me.

Thank you all for accompanying me during the dark and cold days but also the sunny days.

Susanna Maria Lladó Maldonado
Braunschweig, May 2019

Publications of the thesis

This thesis was performed during my doctoral research at Institut für Bioverfahrenstechnik (*ibvt*), Technische Universität Braunschweig, as part of the *EUROMBR* project - *European network for innovative microbioreactor applications in bioprocess development* (Project ID 608104) funded by the People Programme (Marie Curie Actions, Multi-ITN) of the European Union's Seventh Framework Programme for research, technological development and demonstration.

Aspects of this thesis have been published previously:

Chapter 2:

Krull R, Lladó Maldonado S, Lorenz T, Büttgenbach S, Demming S. 2016. Microbioreactors. In: Dietzel A, editor. *Microsystems Pharmatechnology. Manipulation of fluids, particles, droplets, and cells*. Chapter 4, Series Microsystems and Nanosystems, ISBN: 978-3-319-26918-4 (Print) 978-3-319-26920-7 (Online). Cham Heidelberg New York Dordrecht London: Springer International Publishing, pp. 99–152.

Chapter 4:

Lladó Maldonado S, Rasch D, Kasjanow A, Bouwes D, Krühne U, Krull R. 2018. Multiphase microreactors with intensification of oxygen mass transfer rate and mixing performance for bioprocess development. *Biochem. Eng. J.* 139:57–67.

Chapter 5:

Lladó Maldonado S, Panjan P, Sun S, Rasch D, Sesay AM, Mayr T, Krull R. 2019a. A fully online sensor-equipped, disposable multiphase microbioreactor as a screening platform for biotechnological applications. *Biotechnol. Bioeng.* 116:65–75.

Chapter 6:

Lladó Maldonado S, Krull J, Rasch D, Panjan P, Sesay AM, Marques MPC, Szita N, Krull R. 2019b. Application of a multiphase microreactor chemostat for the determination of reaction kinetics of *Staphylococcus carnosus*. *Bioprocess Biosyst. Eng.* 42:953–961.

Articles in Journals

Peterat G, Lladó Maldonado S, Edlich A, Rasch D, Dietzel A, Krull R. 2015. Bioreaktionstechnik in mikrofluidischen Reaktoren. *Chemie Ing. Tech.* 87:505–517.

Lladó Maldonado S, Rasch D, Kasjanow A, Bouwes D, Krull R. 2016. Hydrodynamic and mass transfer characteristics of a microbubble column-bioreactor. *Chemical and Biological Microsystems Society, Dublin*, pp 1483–1484.

Lladó Maldonado S, Rasch D, Kasjanow A, Bouwes D, Krühne U, Krull R. 2018. Multiphase microreactors with intensification of oxygen mass transfer rate and mixing performance for bioprocess development. *Biochem. Eng. J.* 139:57–67.

Lladó Maldonado S, Panjan P, Sun S, Rasch D, Sesay AM, Mayr T, Krull R. 2019a. A fully online sensor-equipped, disposable multiphase microbioreactor as a screening platform for biotechnological applications. *Biotechnol. Bioeng.* 116:65–75.

Lladó Maldonado S, Krull J, Rasch D, Panjan P, Sesay AM, Marques MPC, Szita N, Krull R. 2019b. Application of a multiphase microreactor chemostat for the determination of reaction kinetics of *Staphylococcus carnosus*. *Bioprocess Biosyst. Eng.* 42:953–961.

Chapter Books

Krull R, Lladó Maldonado S, Lorenz T, Büttgenbach S, Demming S. 2016. Microbioreactors. In: Dietzel A, editor. *Microsystems Pharmatechnology. Manipulation of fluids, particles, droplets, and cells*. Chapter 4, Series Microsystems and Nanosystems, ISBN: 978-3-319-26918-4 (Print) 978-3-319-26920-7 (Online). Cham Heidelberg New York Dordrecht London: Springer International Publishing, pp. 99–152.

Oral Presentations at Congresses

Krull R, Lladó Maldonado S, Peterat G. 2015. Reaktionskinetische Untersuchungen zur Chemostatkultivierung von *Saccharomyces cerevisiae* mit einem Mehrphasen-Mikroblasensäulenreaktor. Annual colloquium 2015 DFG SPP 1740 *Reactive bubble flow*, *Honorary colloquium in memoriam Prof. Dr.-Ing. Norbert Rübiger*, TU Hamburg-Harburg, Harburg, August 2015.

Lladó Maldonado S, Krull J, Rasch D, Kasjanow A, Bouwes D, Krühne U, Krull R. 2017. Multiphase microreactors with intensification of oxygen mass transfer rate and mixing performance for bioprocess development, 4th International Conference Implementation of Microreactor Technology in Biotechnology (IMTB 2017), Book of extended abstracts: Session B: Cells within microreactors, BO4, 48 – 49, Bled, Slovenia, April 2017.

Frey LJ, Rasch D, Lladó Maldonado S, Meinen S, Dietzel A, Krull R. 2017. Development of a microbioreactor system for biopharmaceutical applications and analysis of scale down effects. 4th International Conference Implementation of Microreactor Technology in Biotechnology (IMTB 2017), Book of extended abstracts: Session D: Process intensification and integration, DO3, 89 – 90, Bled, Slovenia, April 2017.

Lladó Maldonado S, Rasch D, Frey LJ, Meinen S, Dietzel A, Krull R. 2017. Cultivation at the microscale - development of multiphase microreactors as screening platform for biotechnological and pharmaceutical applications”, 3rd International Symposium on Multiscale Multiphase Process Engineering (MMPE), Proc. L-16, 110-111, Toyama, Japan, May 2017.

Lladó Maldonado S, Rasch D, Kasjanow A, Bouwes D, Krühne U, Krull R. 2017. Bioengineering at the microscale: Process characterization of a microbubble column-bioreactor, 2nd Braunschweig International Symposium on Pharmaceutical Engineering Research – SphERE, Center of Pharmaceutical Engineering, Braunschweig, Germany, September 2017.

Lladó Maldonado S, Krull J, Heydorn RL, Valikhani D, Rasch D, Nidetzky B, Bolivar JM, Krull R. 2017. Cultivation and biotransformation at the microscale: intensification of oxygen mass transfer and mixing in multiphase microreactors, 10th World Congress of Chemical Engineering (WCCE 10) and 4th European Congress of Applied Biotechnology (ECAB4), Barcelona, Spain, October 2017.

Poster Presentations at Congresses

Lladó Maldonado S, Rasch D, Krull R. 2015. Implementation of a CO₂ sensor in microbioreactors, 3rd International Conference Implementation of Microreactor Technology in Biotechnology (IMTB 2015), Book of extended abstracts: Session B: Cells within microstructured devices, BP2, 75 – 76, Opatja, Croatia, May 2015.

Lladó Maldonado S, Sun S, Rasch D, Mayr T, Krull R. 2016. Implementation of optical chemical sensors in a microbubble column-bioreactor, EUROPT(R)ODE XIII, Graz, Austria, March 2016.

Lladó Maldonado S, Rasch D, Kasjanow A, Bouwes D, Krull R. 2016. Hydrodynamic and mass transfer characteristics of a microbubble column-bioreactor, 20th International Conference on Miniaturized Systems for Chemistry and Life Sciences (MicroTAS), Poster M209i, Dublin, Ireland, October 2016.

Lladó Maldonado S, Rasch D, Krull R. 2017. Cultivation at the microscale: Characterization of mixing performance and oxygen transfer in a microbubble column-bioreactor, Spring meeting of the Deutsche Technion-Gesellschaft, Leibnizhaus, Hannover, Hannover, Germany, March 2017.

Frey L, Rasch D, Lladó Maldonado S, Meinen S, Dietzel A, Krull R. 2017. A novel microscale cell culturing system using electrodynamic mixing for biopharmaceutical application, 3rd International Symposium on Multiscale Multiphase Process Engineering (MMPE), Proc. P-15, 265-266, Toyama, Japan, May 2017.

Heydorn RL, Lladó Maldonado S, Panjan P, Bolivar JM, Valikhani D, Sesay AM, Nidetzky B, Krull R. 2017. Bioconversion at microscale: Application of oxygen dependent enzyme immobilizates in microfluidised bed reactors, 2nd Braunschweig International Symposium on Pharmaceutical Engineering Research – SphERE, Center of Pharmaceutical Engineering, Braunschweig, Germany, September 2017.

Krull J, Lladó Maldonado S, Rasch D, Marques MPC, Szita N, Krull R. 2017. Cultivation of *Staphylococcus carnosus* at micro-scale – Influence of different reactor performances, 2nd Braunschweig International Symposium on Pharmaceutical Engineering Research – SphERE, Center of Pharmaceutical Engineering, Braunschweig, Germany, September 2017.

Ostsieker H, Frey L, Vorländer D, Müller B, Lladó Maldonado S, Rasch D, Mayr T, Krull R. 2018. Innovative microbioreactor applications in bioprocess development, 9th Workshop of Chemical and Biological Micro Laboratory Technology, Elgersburg, Ilmenau, Germany, February 2018.

Presentations at Project Meetings of the *EUROMBR* Consortium

Lladó Maldonado S, Rasch D, Krull R. 2015. Bioengineering at the micro-scale: Enhanced mixing on small scales and cultivation in microfluidic screening bioreactors, EUROMBR Meeting 2, University College of London, United Kingdom, February 2015.

Lladó Maldonado S, Rasch D, Krull R. 2015. Real-time online monitoring of an aerobic batch cultivation in a microbubble column, EUROMBR Meeting 3 and 5, TU Graz, Austria, September 2015.

Lladó Maldonado S, Rasch D, Kasjanow A, Krull R. 2016. Characterization of a highly sensor integrated microbubble column-bioreactor, EUROMBR Meeting 6, TU Braunschweig, Germany, May 2016.

Lladó Maldonado S, Rasch D, Krull R. 2016. Evaluation of the mixing performance in a microfluidic reactor, EUROMBR Meeting 7, Technical University of Denmark, Denmark, September 2016.

Lladó Maldonado S, Rasch D, Krull R. 2017. Cultivation at the microscale – Evolution of a microbubble column-bioreactor for bioprocess development. EUROMBR Meeting 8, Magna Græcia University of Catanzaro, Italy, October 2017.

Student's Theses

Krull J. 2017. Cultivation of *Staphylococcus carnosus* at micro-scale - Influence of different reactor performances, Master thesis (58) Institute of Biochemical Engineering, TU Braunschweig/ Microfluidics Laboratory in Biochemical Engineering, University College London (London, United Kingdom).

Heydorn RL. 2017. Intensified bioconversion at microscale: Application of oxygen dependent enzyme immobilizates in microfluidised bed reactors, Master thesis (59), Institute of Biochemical Engineering, TU Braunschweig/ Institute of Biotechnology and Biochemical Engineering, TU Graz (Graz, Austria).

Abbreviations and Symbols

Abbreviations

<i>BF</i>	borofloat
<i>cμBC</i>	cuvette-based microbubble column-bioreactor
<i>CAD</i>	computer-aided design
<i>CFD</i>	computational fluid dynamics
<i>fps</i>	frames per second
<i>gμBC</i>	glass-based microbubble column-bioreactor
<i>GOx</i>	glucose oxidase
<i>hMBR</i>	horizontal microbioreactor
<i>HF</i>	hydrofluoric acid
<i>HTP</i>	high-throughput processing
<i>HTS</i>	high-throughput screening
<i>I</i>	light intensity
<i>LoC</i>	lab-on-a-chip
<i>MBR</i>	microbioreactor
<i>mSTR</i>	miniaturised stirred tank reactor
<i>MTP</i>	microtiter plate
<i>PB</i>	Prussian blue
<i>PBS</i>	phosphate buffered saline
<i>PDMS</i>	polydimethylsiloxane
<i>PIV</i>	Particle Image Velocimetry
<i>PMMA</i>	polymethyl methacrylate
<i>PtTPTBPF</i>	platinum(II) meso-tetra(4-fluorophenyl)tetrabenzoporphyrin
<i>RGB</i>	Red Green Blue colour model
<i>RMS</i>	Root Mean Square
<i>TAS</i>	total chemical analysis system
<i>THF</i>	tetrahydrofuran
<i>U</i>	unit of enzyme activity ($\mu\text{mol}/\text{min}$)
<i>μBC</i>	microbubble column-bioreactor
<i>μFBR</i>	microfluidised bed reactor