

# 89

## **Advances in Biochemical Engineering/Biotechnology**

**Series Editor: T. Scheper**

**Editorial Board:**

**W. Babel · I. Endo · S.-O. Enfors · A. Fiechter · M. Hoare · W.-S. Hu  
B. Mattiasson · J. Nielsen · H. Sahm · K. Schügerl · G. Stephanopoulos  
U. von Stockar · G. T. Tsao · C. Wandrey · J.-J. Zhong**

# Advances in Biochemical Engineering/Biotechnology

Series Editor: T. Scheper

## Recently Published and Forthcoming Volumes

**Recent Progress of Biochemical and Biomedical Engineering in Japan II**  
Volume Editor: Kobayashi, T.  
Vol. 91, 2004

**Recent Progress of Biochemical and Biomedical Engineering in Japan I**  
Volume Editor: Kobayashi, T.  
Vol. 90, 2004

**Physiological Stress Responses in Bioprocesses**  
Volume Editor: Enfors, S.-O.  
Vol. 89, 2004

**Molecular Biotechnology of Fungal  $\beta$ -Lactam Antibiotics and Related Peptide Synthetases**  
Volume Editor: Brakhage, A.  
Vol. 88, 2004

**Biomanufacturing**  
Volume Editor: Zhong, J.-J.  
Vol. 87, 2004

**New Trends and Developments in Biochemical Engineering**  
Vol. 86, 2004

**Biotechnology in India II**  
Volume Editors: Ghose, T. K., Ghosh, P.  
Vol. 85, 2003

**Biotechnology in India I**  
Volume Editors: Ghose, T. K., Ghosh, P.  
Vol. 84, 2003

**Proteomics of Microorganisms**  
Volume Editors: Hecker, M., Müllner, S.  
Vol. 83, 2003

**Biomethanation II**  
Volume Editor: Ahring, B. K.  
Vol. 82, 2003

**Biomethanation I**  
Volume Editor: Ahring, B. K.  
Vol. 81, 2003

**Process Integration in Biochemical Engineering**  
Volume Editors: von Stockar, U., van der Wielen, L. A. M.  
Vol. 80, 2003

**Microbial Production of L-Amino Acids**  
Volume Editors: Faurie, R., Thommel J.  
Vol. 79, 2003

**Phytoremediation**  
Volume Editor: Tsao, D. T.  
Vol. 78, 2003

**Chip Technology**  
Volume Editor: Hoheisel, J.  
Vol. 77, 2002

**Modern Advances in Chromatography**  
Volume Editor: Freitag, R.  
Vol. 76, 2002

**History and Trends in Bioprocessing and Biotransformation**  
Vol. 75, 2002

**Tools and Applications of Biochemical Engineering Science**  
Volume Editors: Schügerl, K., Zeng, A.-P.  
Vol. 74, 2002

**Metabolic Engineering**  
Volume Editor: Nielsen, J.  
Vol. 73, 2001

# Physiological Stress Responses in Bioprocesses

Volume Editor: S.-O. Enfors

With contributions by

T. Egli · S.-O. Enfors · B. Fahnert · M. Hecker · C. J. Hewitt

F. Hoffmann · H. Lilie · G. Nebe-Von-Caron · P. Neubauer · U. Rinas

A. Rozkov · T. Schweder · L. M. Wick



Springer

*Advances in Biochemical Engineering/Biotechnology* reviews actual trends in modern biotechnology. Its aim is to cover all aspects of this interdisciplinary technology where knowledge, methods and expertise are required for chemistry, biochemistry, micro-biology, genetics, chemical engineering and computer science. Special volumes are dedicated to selected topics which focus on new biotechnological products and new processes for their synthesis and purification. They give the state-of-the-art of a topic in a comprehensive way thus being a valuable source for the next 3–5 years. It also discusses new discoveries and applications.

In general, special volumes are edited by well known guest editors. The series editor and publisher will however always be pleased to receive suggestions and supplementary information. Manuscripts are accepted in English.

In references *Advances in Biochemical Engineering/Biotechnology* is abbreviated as *Adv Biochem Engin/Biotechnol* as a journal.

Visit the ABE home page at <http://www.springeronline.com>

ISSN 0724-6145

ISBN 3-540-20311-7

DOI 10.1007/b10847

Springer-Verlag Berlin Heidelberg New York

Library of Congress Control Number 2004102412

This work is subject to copyright. All rights are reserved, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilm or in any other way, and storage in data banks. Duplication of this publication or parts thereof is permitted only under the provisions of the German Copyright Law of September 9, 1965, in its current version, and permission for use must always be obtained from Springer-Verlag. Violations are liable for prosecution under the German Copyright Law.

Springer-Verlag is a part of Springer Science+Business Media

[springeronline.com](http://springeronline.com)

© Springer-Verlag Berlin Heidelberg 2004

Printed in Germany

The use of general descriptive names, registered names, trademarks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.

Typesetting: Fotosatz-Service Köhler GmbH, Würzburg

Cover: KünkelLopka GmbH, Heidelberg; design & production GmbH, Heidelberg

Printed on acid-free paper

02/3020mh – 5 4 3 2 1 0

---

## Series Editor

Professor Dr. T. Scheper  
Institute of Technical Chemistry  
University of Hannover  
Callinstrasse 3  
30167 Hannover, Germany  
*E-mail: scheper@iftc.uni-hannover.de*

## Volume Editor

Professor Dr. S.-O. Enfors  
Department of Biotechnology  
Royal Institute of Technology  
Roslagstullsbacken 21  
10691 Stockholm, Sweden  
*E-mail: enfors@biotech.kth.se*

## Editorial Board

Prof. Dr. W. Babel  
Section of Environmental Microbiology  
Leipzig-Halle GmbH  
Permoserstrasse 15  
04318 Leipzig, Germany  
*E-mail: babel@umb.ufz.de*

Prof. Dr. S.-O. Enfors  
Department of Biochemistry and  
Biotechnology  
Royal Institute of Technology  
Teknikringen 34  
100 44 Stockholm, Sweden  
*E-mail: enfors@biotech.kth.se*

Prof. Dr. M. Hoare  
Department of Biochemical Engineering  
University College London  
Torrington Place  
London, WC1E 7JE, UK  
*E-mail: m.hoare@ucl.ac.uk*

Prof. Dr. I. Endo  
Faculty of Agriculture  
Dept. of Bioproduative Science  
Laboratory of Applied Microbiology  
Utsunomiya University  
Mine-cho 350, Utsunomiya-shi  
Tochigi 321-8505, Japan  
*E-mail: endo@cel.riken.go.jp*

Prof. Dr. A. Fiechter  
Institute of Biotechnology  
Eidgenössische Technische Hochschule  
ETH-Hönggerberg  
8093 Zürich, Switzerland  
*E-mail: ae.fiechter@bluewin.ch*

Prof. W.-S. Hu  
Chemical Engineering and Materials Science  
University of Minnesota  
421 Washington Avenue SE  
Minneapolis, MN 55455-0132, USA  
*E-mail: wshu@cems.umn.edu*

**Prof. Dr. B. Mattiasson**

Department of Biotechnology  
Chemical Center, Lund University  
P.O. Box 124, 221 00 Lund, Sweden  
*E-mail: bo.mattiasson@biotek.lu.se*

**Prof. Dr. H. Sahlm**

Institute of Biotechnology  
Forschungszentrum Jülich GmbH  
52425 Jülich, Germany  
*E-mail: h.sahlm@fz-juelich.de*

**Prof. Dr. G. Stephanopoulos**

Department of Chemical Engineering  
Massachusetts Institute of Technology  
Cambridge, MA 02139-4307  
USA  
*E-mail: gregstep@mit.edu*

**Prof. Dr. G. T. Tsao**

Director  
Lab. of Renewable Resources Eng.  
A. A. Potter Eng. Center, Purdue University  
West Lafayette, IN 47907, USA  
*E-mail: tsaogt@ecn.purdue.edu*

**Prof. Dr. J.-J. Zhong**

State Key Laboratory  
of Bioreactor Engineering  
East China University of Science  
and Technology  
130 Meilong Road  
Shanghai 200237, China  
*E-mail: jjzhong@ecust.edu.cn*

**Prof. J. Nielsen**

Center for Process Biotechnology  
Technical University of Denmark  
Building 223, 2800 Lyngby, Denmark  
*E-mail: jn@biocentrum.dtu.dk*

**Prof. Dr. K. Schügerl**

Institute of Technical Chemistry  
University of Hannover, Callinstraße 3  
30167 Hannover, Germany  
*E-mail: schuegerl@iftc.uni-hannover.de*

**Prof. Dr. U. von Stockar**

Laboratoire de Génie Chimique et  
Biologique (LGCB), Département de Chimie  
Swiss Federal Institute  
of Technology Lausanne  
1015 Lausanne, Switzerland  
*E-mail: urs.vonstockar@epfl.ch*

**Prof. Dr. C. Wandrey**

Institute of Biotechnology  
Forschungszentrum Jülich GmbH  
52425 Jülich, Germany  
*E-mail: c.wandrey@fz-juelich.de*

---

## **Advances in Biochemical Engineering/Biotechnology Also Available Electronically**

For all customers who have a standing order to *Advances in Biochemical Engineering/Biotechnology*, we offer the electronic version via SpringerLink free of charge. Please contact your librarian who can receive a password for free access to the full articles by registering at:

<http://www.springerlink.com>

If you do not have a subscription, you can still view the tables of contents of the volumes and the abstract of each article by going to the SpringerLink Homepage, clicking on "Browse by Online Libraries", then "Chemical Sciences", and finally choose *Advances in Biochemical Engineering/Biotechnology*.

You will find information about the

- Editorial Board
- Aims and Scope
- Instructions for Authors
- Sample Contribution

at <http://www.springeronline.com> using the search function.

---

## **Attention all Users of the “Springer Handbook of Enzymes”**

Information on this handbook can be found on the internet at  
**<http://www.springeronline.com>**

A complete list of all enzyme entries either as an alphabetical Name Index or as the EC-Number Index is available at the above mentioned URL. You can download and print them free of charge.

A complete list of all synonyms (more than 25,000 entries) used for the enzymes is available in print form (ISBN 3-540-41830-X).

# Save 15 %

We recommend a standing order for the series to ensure you automatically receive all volumes and all supplements and save 15% on the list price.



---

## Preface

The physiological stress responses are universal phenomena that have been developed by all kinds of cells as a defence to harmful environmental conditions. Thus, it is often in the environmental microbiology literature that one finds information on microbial stress responses. However, a bioreactor with growing cells can also provide conditions that are experienced by the cell as stress and to which it responds by altering its metabolism and physiology. Even if a brief look into a large industrial bioreactor gives an impression of good mixing and homogeneity and the data logging of on-line control parameters gives the impression of slow trends or close to steady-state conditions, dramatic physiological responses take place in the cell. It can therefore be expected that the physiological stress responses have a large influence on the process outcome.

In a previous EU-project [1], a number of scale-up responses were observed when the performance of an *E. coli* strain was compared in lab scale and industrial 10–30 m<sup>3</sup> bioreactors [2]. These included, differences in biomass yield, recombinant protein accumulation, and cell viability. It was assumed that a major reason for these scale-up effects were related to metabolic and stress responses induced by the glucose and oxygen concentration gradients in large bioreactors and this gave rise to the idea of reviewing microbial stress responses with special reference to the situation in bioreactors.

This volume of *Advances in Biochemical Engineering and Biotechnology* reviews a number of stress issues that are relevant to bioprocessing. The examples are selected mainly with respect to *Escherichia coli* and the production of recombinant proteins. However, the universal character of physiological stress makes much of the information generally valid for bioprocessing even if details in the stress response machinery differ between prokaryotes and eukaryotes.

The first chapter reviews the molecular components involved in the stress responses and how these responses are aimed at protecting the cell from certain threats. One stress agent that is certainly man-made and of a recent date is the synthesis and accumulation of recombinant proteins in a cell. Obviously, the response to the stress such “abnormal” proteins induce was developed long time ago, but it has a large impact on today’s bioprocessing. The third chapter describes the formation and utilisation of inclusion bodies, which can be considered as abnormal protein aggregates in the cell which are often encountered when a cell is forced to produce a recombinant protein. Also for this situation, a molecular stress handling machinery has been developed, involving, among other components, the so called chaperones that assist in protein folding. Final-

ly, three chapters are devoted to different aspects of the analysis of stress responses or stressed cells at different levels: the gene expression, the proteolytic degradation of “unwanted” proteins, and the “health status” of the cells, as monitored by flow cytometry.

Stockholm, March 2004

Sven-Olof Enfors

## References

1. Bioprocess Scale-up Strategy Based on the Integration of Microbial Physiology and Fluid Dynamics. EU Project No: BIO4-CT95-0028
2. Enfors S-O, Jahic M, Rozkov A, Xu B, Hecker M, Jürgen B, Krüger E, Schweder T, Hamer G, O’Beirne D, Noisommit-Rizzi N, Reuss M, BooneL, Hewitt C, McFarlane C, Nienow A, Kovacs T, Trägårdh C, Fuchs L, Revstedt J, Friberg PC, Hjertager B, Blomsten G, Skogman H, Hjort S, Hoeks F, Lin H-Y, Neubauer P, van der Lans R, Luyben K, Vrabel P, Manelius Å (2001) Physiological responses to mixing in large scale bioreactors. *J Biotechnol* 85:175–185

---

# Contents

<b>Molecular Components of Physiological Stress Responses in <i>Escherichia coli</i></b> L. M. Wick · T. Egli . . . . .	1
<b>Monitoring of Stress Responses</b> T. Schweder · M. Hecker . . . . .	47
<b>Stress Induced by Recombinant Protein Production in <i>Escherichia coli</i></b> F. Hoffmann · U. Rinas . . . . .	73
<b>Inclusion Bodies: Formation and Utilisation</b> B. Fahnert · H. Lilie · P. Neubauer . . . . .	93
<b>Roles of Heat-Shock Chaperones in the Production of Recombinant Proteins in <i>Escherichia coli</i></b> F. Hoffmann · U. Rinas . . . . .	143
<b>Analysis and Control of Proteolysis of Recombinant Proteins in <i>Escherichia coli</i></b> A. Rozkov · S.-O. Enfors . . . . .	163
<b>The Application of Multi-Parameter Flow Cytometry to Monitor Individual Microbial Cell Physiological State</b> C. J. Hewitt · G. Nebe-Von-Caron . . . . .	197
<b>Author Index Volumes 51–89</b> . . . . .	225
<b>Subject Index</b> . . . . .	241