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Bioanalysis and Biosensors for Bioprocess Monitoring

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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, microbiology, 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 managing editor and publisher will however always be pleased to receive suggestions and supplementary information. Manuscripts are accepted in English.

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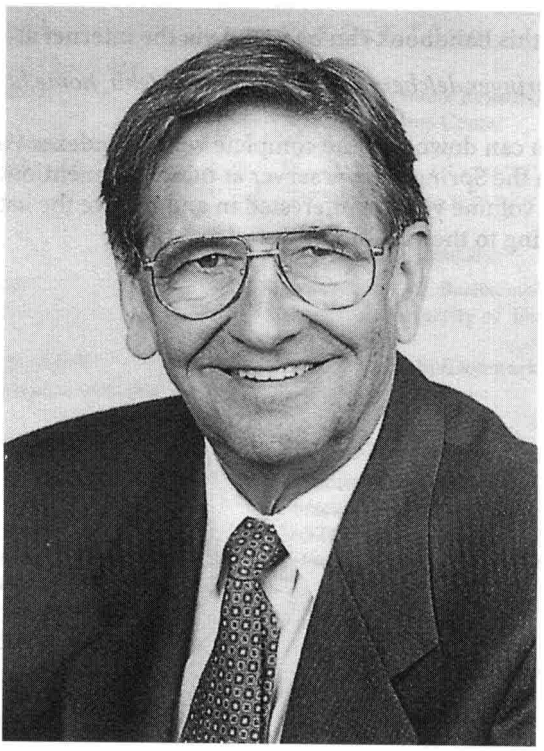

Professor Dr. A. Fiechter

Laudatio

This volume is dedicated to Dr. Armin Fiechter, Professor Emeritus of Biotechnology at the ETH Zürich and former managing editor of Advances in Biochemical Engineering/Biotechnology and Journal of Biotechnology and editor and member of Advisory Boards of several international periodicals on the occasion of his 75th birthday.

Armin Fiechter is one of the pioneers in biotechnology – recognized worldwide for his important contributions to various fields of biotechnology. Professor Fiechter's research covers a broad area. He carried out pioneering work in several fields. From the beginning, he stressed the necessity of interdisciplinary and international cooperation. He especially promoted cooperation between engineering and biological research groups and helped to overcome the hurdles and borders between these groups. His active role as a teacher of young scientists led to the well known "Fiechter School". Some well-known researchers in industry and science come from his laboratory. His more than 500 publications document his research activities in different areas of biotechnology.

The quantitative evaluation of biological regulation was especially difficult, because reproducibility of the measurement of the dynamical processes was unsatisfactory in the 1960s. One of the first long-term continuous cultivation of baker's yeast in a chemostat system in combination with aseptic operation and use of pH- redox- and oxygen-electrodes was realized by his group. The sterility was obtained by O-ring sealing. The sterilizable pH-, redox- and oxygen electrodes were developed in the industry with his co-operation. The sealing of the stirrer shaft with a sliding sleeve and the use a marine propeller in combination with a draft tube (compact loop reactor, COLOR) for maintaining ideal mixing and for better mechanical foam control was also developed in cooperation with his group. One of the key issue was the better process control by means of in situ monitored pH- and redox-values and dissolved oxygen concentration in the cultivation medium under aseptic operation. Various instruments (FIA, HPLC, GC, MS) were adapted for on-line monitoring of the concentrations of key components and computer programs were developed for automatic data evaluation and control. In this compact loop reactor and by means of advanced measuring and control systems highly reproducible measurements became possible.

Professor Fiechter succeeded to show using the improved chemostat technique that glucose and oxygen influence various yeast stains differently. Beside the catabolite repression (glucose effect) a second regulation type exists which is controlled by the dynamic substrate flux (glucose). This causes different types

of physiological phenomena such as diauxie, secondary monoauxie or atypical changes in growth and ethanol production continuous cultures. Sonnleitner and Kaeppeli in his group developed an overflow model to explain these phenomena. Overflow reaction is common not only in yeast, but in bacteria as well. In addition, they investigated the cell cycle by means of the analysis of stable synchronous growth, which was maintained in the high performance chemostat system. It was possible to recognize the trigger-function of trehalose for the onset of budding and the testing of the secretion and reuse of metabolites during the budding.

Investigations of the processes with different strains and reactor types under close control are necessary for the transfer of biological processes from a laboratory to an industrial scale (scale up). Most of the early biochemical engineering research was restricted to the investigation of oxygen transfer and carried out with model media without micro-organisms. Systematic pilot plant investigations were performed with various micro-organisms and different types of reactors up to 3000 l volume in Hönggerberg by the Fiechter research group. The reactor performances were compared and optimal process operations were evaluated. The high process performance of the compact loop reactor was proved.

In addition to this technical oriented development, a broad field of applied biological research was at the center of interest in Fiechter's laboratory. The development of bioreactors, bioprocess monitoring and control served as a means of obtaining more information on the biology of microorganisms and improving the process performance.

The investigation of the physiology of baker's yeast was a central issue in this laboratory. Evaluation of the details of the cell cycle and the importance of the overflow phenomenon are discussed above. However, other microorganisms, such as the strictly respiratory yeast, *Trichosporon cutaneum*, and bacteria, such as *Escherichia coli*, were investigated and applied for reactor characterization as well. *Zymomonas mobilis* surpasses baker's yeast with regard to alcohol production by a factor of five. In the high performance reactor under aseptic conditions extremely high ethanol productivities ($250\ ml\,l^{-1}\,h^{-1}$) were obtained in Fiechter's laboratory.

As early as 1983, a cell culture group was established and in the following 10 years serum- and protein-free cultivation media were developed by means of a systematic analysis of key C-sources, intermediate and final metabolites and their influence on the growth and product formation. Lactate formation was identified as an overflow phenomenon caused by a respiratory bottleneck, incomplete medium composition, glucose excess, and stress factors. In continuous cultivation of CHO cells with cell recycling generation times of 12 h were obtained. By means of a Process Identification and Management System (PIMS), which was developed by his group, automatic on-line analysis and control of animal tissue cultivation became possible. In cooperation with Weissmann, recombinant Interferon was produced by *Escherichia coli* in a 3000 l reactor for clinical investigations in 1980.

Of his many research activities only few have been mentioned: In the frame of the SCP project, Cytochrome P-450 studies were carried out in connection

with the investigation of hydrocarbon metabolisms of yeasts. Enzymes from thermophilic bacteria (*Bac. stearothermophilus*) were identified and isolated. In connection with biodegradation of lignin, new enzymes were identified and isolated. In the framework of the microbial-enhanced oil recovery project Rhamnolipid biotensides were produced by genetically modified *Pseudomonas aeruginosa*. A process for the production of Lipoteichonacid (LTA) was developed and the anticarcinogenic compound was produced in a 3000 l reactor. Outside of industry, no other academic research group gained so many important results on the pilot plant scale. These and many other results help us in transferring biotechnological processes from the laboratory to the industrial scale.

Because of his broad spectrum of activities and successful research he was invited into several countries and where he acted as visiting professor. He became a member of the Supervisory Board of GBF (Central Biotechnology Research Laboratory of Germany), Braunschweig, a member of the Board and Interim Director of the Institute of Surface- and Biotechnology of the Fraunhofer-Society, Stuttgart, a member of the Swiss Academy of Engineering Sciences, a founding member of the European Federation of Biotechnology, a member of the IUPAC Commission on Microbiology, an honorary member of DECHEMA, president of the Swiss Microbial Society, etc.

We, his colleagues and former students thank him for his enthusiasm and continuous support in biotechnology also after his retirement. By dedicating this volume of *Advances in Biochemical Engineering/Biotechnology* to Professor Fiechter, the authors of this volume and many other colleagues around the world want to honor his outstanding achievements in the broad field of biotechnology and wish him good health.

Hannover, July 1999 Karl Schügerl

Preface

This special volume on "bioanalysis and biosensors for bioprocess monitoring" has a twofold target.

Firstly, it is dedicated to the 75th birthday of Armin Fiechter, who was a major driving force among the pioneers to the progress of biochemical engineering. Not only the aseptic connection technique with septa and needles still used until today was established by him, but also the development of the first sterilizable pH-electrodes with W Ingold is also credited to him. He made in-vivo bioanalysis a topic of general interest, for instance by setting up the first chemostat in Switzerland. It was again Armin Fiechter who pushed the use of non-invasive exhaust gas analysis in the late 1960s and promoted development and exploitation of in-situ sensors and on-line analytical instruments in bioprocessing, among other means, by founding a spin-off company. In his laudatio, Karl Schügerl extends the list of his merits and achievements.

On the other hand, this volume is the first product of a core group working in the first Task Group "synopsis of conventional and non-conventional bioprocess monitoring" of the first Section of the EFB, namely the Section on Biochemical Engineering Science. All the various monitoring techniques are so determinant and central that the EFB decided to found the Working Party on Measurement and Control, as one of the last Working Parties, as late as 1988. The Section, however, was founded in 1996 in order to facilitate communication and cooperation among biochemical engineers and scientists so far organized, or should I say split up, into various different Working Parties. It was strongly felt that the business of measurement (modeling) and control could not be confined to the respective Working Party, it was and is so important for all the colleagues associated with bioreactor performance or down stream processing that a broadening of the horizon was actively sought.

Within the Section, several Task Groups are playing the role of workhorses.

A synopsis of monitoring methods and devices was missing from the beginning. The interest in obtaining up-to-date information and exchanging mutual experience with older and up-to-date bioprocess monitoring tools became obvious before, during and after several advanced courses organized and run by the predecessors of the present Section. The conclusion soon became clear, but the realization came later, and here is the first report from the Task Group!

Certainly, these few contributions cover a great variety of achievements, bring some success stories, discuss some potential pitfalls and discuss several practical experiences. It is clear that this synopsis is non-exhaustive; it is also obvious

that we have failed to include contributions specifically focused on downstream processing and product qualification problems or targeted to bioreactor performance characterization. However, it was important to show, with a first report, that there are people active in these fields and, hopefully, continuing to be so and attracting more people to join them in this work.

The contributions to this special volume were selected in order to show the present dynamics in the field of bioprocess monitoring. Some quite conventional methods are addressed, other contributions focus on more fuzzy things such as electronic noses or chemometric techniques. One contribution illustrates the potential with a precise example of cephalosporin production. Three of them have dared to "look" inside cells using different methods, one by the analysis of (microscopic) images, one by trying to estimate the physiological state, and the third by analyzing the metabolic network. This gives a rough but good idea of how sophisticated analytical tools – (bio)chemical ones hand in hand with mathematical ones, – give rise to a better understanding of living systems and bioprocesses.

Along with monitoring and estimation we also focus on modeling and control of bioprocesses in the future. Perhaps, other Task Groups will evolve to accomplish this. In the field of monitoring and estimation, we face the great challenge of realizing an appropriate technology transfer of many scientific highlights described in this volume into everyday industrial applications. A big gap in knowledge and experience still makes the decision between "must" and "nice to have" not easy. I hope that this special volume initiates many successful steps towards this goal.

Winterthur, June 1999 B. Sonnleitner

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Instrumentation of Biotechnological Processes

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Modern bioprocesses are monitored by on-line sensing devices mounted either in situ or externally. In addition to sensor probes, more and more analytical subsystems are being exploited to monitor the state of a bioprocess on-line and in real time. Some of these subsystems deliver signals that are useful for documentation only, other, less delayed systems generate signals useful for closed loop process control. Various conventional and non-conventional monitoring instruments are evaluated; their usefulness, benefits and associated pitfalls are discussed.

Keywords. Conventional and non-conventional sensors and analytical instruments, On-line bioprocess monitoring, Software sensors, Dynamics of measurements, Real time estimation, Interfacing aseptic sampling

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