Mass Spectrometry in Biotechnological Process Analysis and Control

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PREFACE

This book is based on the contributions to the IFAC-Workshop "Mass Spectrometry in Biotechnological Process Analysis and Control" held in Graz, Austria from 23 to 24 October 1986.

The idea to organize this workshop and further to prepare these proceedings was stimulated by the following facts. Biotechnological processes urgently need better on-line instrumentation. Mass spectrometry (MS) offers a great potential to especially analyse gases and volatile compounds. It is, however, considered that this potential by far is not exhausted. The main reason for this is that MS often still is considered to be a very expensive technique requiring the permanent attention of a MS expert. In addition methods have not yet been developed to a user friendly state.

On-line MS-methods are available to a certain extent, but need further development. To stimulate such development an interdisciplinary effort is necessary. Needs of industrial and university users and experience of physicists and instrument manufacturers have to be brought into a hopefully fruitful discussion. An introductory article describes the bioprocess background including a brief summary of the state of the art in bioprocess sensor and parameter estimation development, and the potential MS offers for bioprocess monitoring.

In the first chapter on "Instrumentation and Gas Analysis" a general overview on some developments in MS-instrumentation is given initially by Schmid. Then the presently available instrumentation for bioprocess monitoring is discussed by instrument manufacturers (Winter; Schaefer and Schultis; Bartman). This instrumentation mainly involves process gas analysis, but liquid phase analysis using membrane interfaces has been recently offered too. In the field of gas analysis industrial applications are rather common today.

The second chapter "Membrane Inlet Systems" discusses some of the problems of such inlet systems (Cox; Griot et al.). In some cases the dynamic response is not satisfactory. The signal intensity may be influenced by a number of factors including

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membrane characteristics and vacuum tube connection to the MS. This is especially true in the case of monitoring volatiles. Monitoring of dissolved gases is possible also in situations where no other sensors give satisfactory results (e.g. hydrogen, Lloyd et al.). A general discussion on gas analysis and membrane probes is given at the end of this chapter.

The third chapter on "Applications and Computer Control" gives several examples on various applications. Bohatka et al. describe their multiprobe system for gas and liquid phase analysis in a pharmaceutical company. MS was also used to characterize gas residence time distribution and liquid phase mixing using gas and volatile trace compounds (Luebbert et al.). MS was used to get a detailled view on the dynamic behaviour of anaerobic digesters and even to control such reactors based on dissolved hydrogen (Whitmore et al.). Richards et al. used MS to monitor several gases during microbial oxidation of gaseous hydrocarbons. Van Tilborg used GC-MS techiques to determine the production of minute amounts of ethanol in the presence of large amounts of that volatile.

In the last chapter on "Pyrolysis-MS and HPLC-MS Interfacing" methods to analyse also less volatile compounds are discussed. Such methods could give valuable information about almost any bioprocess. Boon et al. show how Curie point pyrolysis combined with MS and with GC-MS could be used to differentiate between phage sensitive and phage resitant strains. These methods also allowed a deeper insight into cell wall composition. Sandmeier et al. describe their attempt to establish a robust method for on-line pyrolysis MS. Lankmayr gives a brief review on HPLC-MS interfacing techniques. Finally in a discussion on "Mass Spectrometry for Control of Fermentation" the general value of on-line MS is discussed.

It is the hope of the organizers of the workshop and editors of this book to have helped in stimulating the interdisciplinary discussion to develop new useful MS methods and instruments for bioprocess monitoring.

Elmar Heinzle

ACKNOWLEDGEMENTS

The success of this workshop on "Mass Spectrometry in Biotechnological Analysis and Control" which was held in Graz, Austria from 23 to 24 October 1986 was made possible by the fortunate conjunction of support and cooperation of many institutions and individuals. The workshop was organized by the Institute for Environmental Research of the Joanneum Research Society, which is setting up a biotechnology center. It was sponsored by the International Federation of Automatic Control (IFAC) and the IFAC Committee on Applications (APCOM). It was only possible to organize this meeting with the generous financial support by a number of companies producing mass spectrometer equipment (Balzers AG, Liechtenstein; Extrel Corporation, Pittsburgh PA, U.S.A.; Klaus Schaefer GMBH, Langen, FRG; Leybold-Heraeus GmbH, Koeln, FRG and VG Instruments AG, Wiesbaden, FRG) and by the governments of Styria and Graz.

The program was set up by the International Program Committee consisting of J.J. Boon, C.L. Cooney, R.P. Cox, A. Halme, E. Heinzle, D. Lloyd, E. Pungor Jr., M. Reuss, and K. Schuegerl. We acknowledge the valuable work that was done by the National Organizing Committee (H. Esterbauer, E. Heinzle, R.M. Lafferty and R. Pinther-Scheuer).

We express thanks to Plenum Press who made it possible to publish these proceedings. We especially thank W. Pritz for transfering discussions from tape into a readable form on paper. We appriciate the cooperation with M. Carter and J. Matzka and their associates from Plenum Press made it very much easier for us to finish these proceedings. We also express our thanks to S. Klambauer and J. Brandl for doing most of the typing work.

Most importantly, the success of this workshop rested upon the contribution of the speakers, convenors and author whose efforts in carefully preparing their manuscripts and whose vitality in discussing the topic throughout the meeting we enthusiastically acknowledge in preparing this volume.

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APPLICATIONS AND COMPUTER CONTROL

PYROLYSIS-MS AND HPLC-MS INTERFACING

INTRODUCTION

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BIOCHEMICAL PROCESS MONITORING AND CONTROL

Biotechnological processes are no longer an art form, using only the knowledge of experienced operators and are now more likelyto use engineering disciplines. These include reaction engineering, process design, and process control.

Biotechnological process investigation and operation is usually very expensive. Most of the processes are rather slow often lasting a couple of days and aseptic operation is absolutely necessary to avoid competition by undesirable organisms. These and other reasons make it very desirable to get as much information as possible out of each experiment and to closely monitor or even control production processes to minimize costs and optimize product quality. Product quality may be improved by increased selectivity, increased product concentration and increased reproducibility which significantly may reduce costs for down-stream processing.

A necessary prerequisit for process control is the on-line measurement of important process variables. As most of the process variables presently cannot be measured on-line, there is a great need for such measurement methods. Two important reasons for such optimal control and therefore for monitoring are:

- A number of products is now produced under strong commercial competition (e.g. penicillin). To reduce costs process conditions (yield, rates, reproducibility, ...) have to be optimized.

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- Many processes are run in a fed batch mode. Optimal feed strategies require feed back control, which essentially needs relevant information of the actual process behaviour.

From present trends and expected future requirements it is evident that process development increasingly will include development of process analysis and control as an integral part. To improve process analysis, developments in mainly two directions are necessary. Firstly, new analytical methods have to be applied, modified, or even newly developed. Secondly, existing information (e.g. heat production, gas analysis, torque measurement, etc.) in combination with modelling can give information on interesting process variables.

These needs have been realized by a number of companies and research groups and recently have led to two specialized symposia:

- "Analytical Methods in Biotechnology", 1984, Noordwijkerhout, The Netherlands. Most contributions were published in an issue of Analytica Chimica Acta, Vol. 163.
- "Modelling and Control of Biotechnological Processes", 1985, Noordwijkerhout, The Netherlands. Papers were published by Pergamon Press, Oxford, 1985 (Ed. A. Johnson).

Continuously Operating Sensors

In many cases batch or continuous sampling and subsequent analysis may be sufficient. But obviously the most desirable method for process monitoring is the application of continuously operating sensors. These should have a number of characteristics:

- The specificity should be high enough to give a useful signal for the concentration of one single chemical species or a group of them.
- Because of low concentrations especially at the initial state of process development the sensitivity often must be very high.
- In most cases steam sterilizability will be a necessary precondition.
- The sensor should not interfere with the process.
- Accuracy requirements often will be moderate (1 - 10 % relative).
- Long term stability will reduce the need for recalibration, which under sterile conditions anyway would be very cumbersome.
- Sensor costs and maintenance requirements may be practical limits for certain applications.
- Dynamic characteristics will play an important role if the measured variable changes rather quickly.

To control a process the sensor will be an integral part of the feed-back control loop. The quality of the sensor must be related to the process characteristics and the requirements for control performance.

There are already a number of sensors that are used in bioprocess monitoring. These, however, are mostly sensors for physical variables or are not directly steam sterilizable (pH, redox potential, dissolved oxygen and CO_2, penicillin, glucose, etc). Well established methods are measurement of temperature, pressure, pH, etc. Only very few sensors directly can measure product or substrate concentration under sterile conditions. Biomass concentration or activity usually cannot be measured on-line.

Parameter Estimation

If the process is completely a black box, i.e. if no descriptive model exists, the variable to be controlled to desirable values must be measured directly. If some characteristics of the process are known, a model can be built which eventually allows the measurement and control of a related variable to finally control the desired variable. Estimators have been applied to allow calculation of interesting process variables. Unfortunately, in biotechnical processes the knowledge of the process mechanistics often is very limited. On-line estimation of variables often may be improved considerably by applying filter techniques.

Present Trends in Developments of Process Analysis

Present trends towards the development of new methods or improving existing ones can be classified as follows.

- Improvement in sampling techniques to couple powerful analytical methods to processes (HPLC, enzymatic sensors, automatic chemical analysis).
- Application of chip production technology to develop new miniaturized sensors. These may be multicomponent sensors, not steam sterilizable but very cheap. They can be used in very small amounts of sample stream to continuously monitor a series of variables in the non sterile region of a process.
- Adaptation of existing analytical methods (fluorimetry, laser technology, mass spectrometry, infrared spectroscopy.
- Application of balancing methods to calculate new variables from available sensor signals.
- Incorporation of suitable mathematical filter techniques may further improve calculated variable accuracy.

One of the most promising fields in adaptation of existing analytical methods isclaimed to be mass spectrometry.

MASS SPECTROMETRY APPLICATIONS IN VARIOUS FIELDS

One outstanding advantage of mass spectrometry (MS) ist that it is fairly general and also specific detector. MS has a wide linear measurement range over several orders of magnitude.

There are, however, also some limitations mainly in the sample introduction. Gases and volatiles can be most easily introduced into the high vacuum and ionized therein. Most of the biotechnologically interesting compounds are non-volatiles. Analysis of these compounds is more difficult but generally possible. Using plasma desorption, MS protein molecules with molecular weights of more than 40.000 have been analysed. Another limitation of MS is in the analysis of mixtures because of the overlapping of individual spectra. This is the reason for coupling chromatographic processes in front of MS detection. To a certain extent soft ionization methods may be used to simplify spectra for direct mixture analysis. Another disadvantage may be the complexity of MS instrumentation and its high costs. This obviously is changing now with the introduction of small instruments with cheap microcomputers.

MS has a long history having been developed since the beginning of this century. First quantitative gas analysis of volatile hydrocarbons was carried out in 1940. In 1949 MS was already successfully applied to breath analysis and later in chemical process monitoring. Breath analysis still is an important field of on-line MS application. Speed of detection and the inherent ability of MS to simultaneously analyze several gaseous compounds are the main advantages in this case. The application of MS for analysis of steel processes to monitor the progress of oxidation of carbon is a very well established method . This example shows that MS can be used in harsh industrial environment. In chemical processes MS may be superior to gas chromatography and other methods when speed of analysis is crucial e.g. in the production of ethylene oxide, propylene oxide, acrylonitrile, vinyl acetate and vinylchloride or when sampling has to be done at many points.

In medical areas apart from breath analysis a great deal of effort has been applied to blood gas monitoring using membrane probes. Monitoring of blood gases and volatile anaesthetics may be very attractive during critical operations. More recently non-invasive transcutaneous measurement was used to detect dissolved oxygen and helium in arterial blood using a specially designed membrane probe.

MS also has been applied in monitoring of atmospheric pollution. Process MS has a wide application in control of submarine atmosphere. MS is ideally suited for monitoring stable isotopes to avoid radiation hazards. Most interesting isotopes are ^{2}H, ^{13}C, ^{15}N, ^{17}O and ^{18}O.

MS has also been applied in direct analysis of complex mixtures though it is inherently limited in this respect because of overlapping of peak fragments of individual components. Despite this limitation, MS was successfully used to continuously analyse natural gas samples. MS was coupled to pyrolysis ovens to analyze coal, to classify microorganisms, to analyze polymers, polymer mixtures and analyse other complex biological samples. Recently a process MS using chemical ionization has been designed and applied to the analysis of complex environmental samples containing chlorinated compounds and heavy metals.

MS IN BIOTECHNOLOGICAL PROCESS ANALYSIS

It seems to be clear that MS offers a great potential for the analysis of a number of interesting compounds in biotechnological processes. Several papers describing the application of MS in biotechnological process analysis have been published and also reviewed recently.

Despite the fact that research and development of on-line measurement methods for monitoring the state of biotechnological processes have been well presented at various conferences related to biotechnology there are good reasons to additionally discuss specific problems of individual measurement techniques in the environment of special workshops. As a matter of fact, in view of the exploding sizes of the various national and international conferences dedicated to the entire field of biotechnology specialized workshops seem to be more and more indispensable to keep a creative atmosphere for scientific discussions. Such discussions, including the exchange of experiences can be extremely useful particularly in the area of appliation of sophisticated measurement techniques because the expenses for obtaining ones own experience is immense and may easily grow beyond the limits of the individual research laboratories and smaller companies.

Related to the application of MS-methods for the on-line analysis of fermentation processes it is particularly important, and this was also the intention of organizing this workshop, to bring together research groups developing and applying MS-methods in biotechnology, industrial suppliers of MS equipment, and industrial users of MS to summarize the present knowledge in this field, to transmit the knowledge about the potential and limitations of MS-methods to users, and to make knoen the needs of users. We think there were a number of interesting contributions and vivid discussion in the plenum and during the meeting. We

hope that publishing these papers and discussions will help to distribute this material to an extended group of interested people and that it helps to stimulate developments in this field.

The IFAC-Workshop "Mass Spectrometry in Biotechnological Process Analysis and Control", was organized by the Institut for Environmental Research, Graz, Austria and attracted about 60 people from European contries and from the U.S.A.

Most of the contributions given during the workshop are contained in these proceedings.

DEVELOPMENTS IN MASS SPECTROMETRIC INSTRUMENTATION RELEVANT TO BIOTECHNOLOGY

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INTRODUCTION

In many disciplines the successful application of Mass Spectrometry (MS) has steadily increased. This holds also true for biotechnology. The reason for this is that mass spectrometry can be applied to all elements and to molecules with molecular weights greater than 15.000 dalton. The response is linear over many orders of magnitude, and it is an extremely sensitive method which needs only very small sample amounts.

The basis of mass spectrometry is the measurement of mass, one of the two fundamental quantities to characterize matter. The second one is the frequency, the basis of other analytical methods like UV-, IR- spectrometry, X-ray or NMR-spectrometry. Mass spectrometry has the great advantage over these methods that much less substance is needed, roughly thousend times less e.g. in comparison to NMR-spectrometry, which can be otherwise equally powerful in identifying or characterizing the structure of organic substances.

Especially the high information content of a mass spectrum combined with the high sensitivity and therefore low sample amount needed is responsible for the fast dissemination of this method in spite of the relatively high costs for the instrumentation. Furthermore, it is of advantage to be able to determine several substances at a time with a mass spectrometer in contrast to only one substance or one parameter in the case of some other instruments.

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