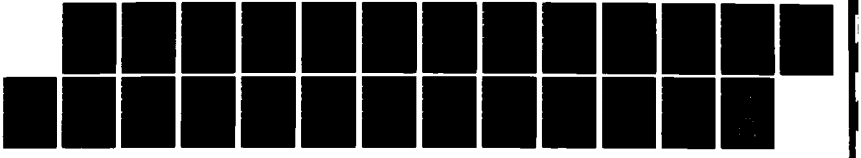
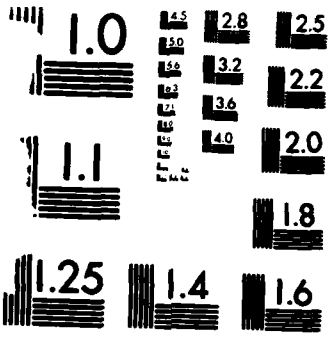
AD-A165 415 BIOTECHNICA '85 INTERNATIONAL CONGRESS FOR BIOTECHNOLOGY HELD IN HANNOVER (U) OFFICE OF NAVAL RESEARCH LONDON (ENGLAND) C E ZOMZELY-NEURATH 1/1

UNCLASSIFIED 27 FEB 86 ONRL-C-13-85 F/G 6/1 NL





(12)

ONRL Report C-13-85

AD-A165 415

Biotechnica '85 1st International Congress for Biotechnology, Hannover, West Germany

Claire E. Zomzely-Neurath

27 February 1986

Approved for public release; distribution unlimited

U.S. Office of Naval Research, London

UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PAGE

AD-A165415

REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT DISTRIBUTION STATEMENT A Approved for public release; Distribution Unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) C-13-85	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION US Office of Naval Research Branch Office, London 6b. OFFICE SYMBOL (If applicable) ONRL	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code) Box 39 FPO, NY 09510	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (City, State, and ZIP Code)	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. / PROJECT NO. / TASK NO. / WORK UNIT ACCESSION NO.

11. TITLE (Include Security Classification)
Biotechnica '85 1st International Congress for Biotechnology, Hannover, West Germany

12. PERSONAL AUTHOR(S)
Claire E. Zomzely-Neurath

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Conference	FROM _____ TO _____	27 Februry 1986	21

16. SUPPLEMENTARY NOTATION

17 COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Biocatalysts, Modeling, Process control, Cell cultures
06	01/06/12		

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

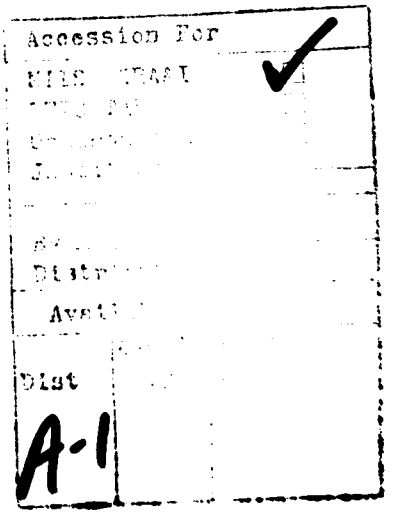
This report provides a review in some detail of the presentations in the three general areas: measurement of process control and development of models; biocatalyst preparation, utilization, and improvement; and animal and plant cell cultures. The report concludes that the excellent presentations showed that biotechnology research in Europe and the UK is of high caliber and represents a greatly increased emphasis on basic as well as applied research in biotechnology.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	UNCLASSIFIED
22a. NAME OF RESPONSIBLE INDIVIDUAL C.J. Fox	22b. TELEPHONE (Include Area Code) (44-1) 409-4340
	22c. OFFICE SYMBOL 11

DD FORM 1473, 84 MAR 83 APR edition may be used until exhausted
All other editions are obsolete

SECURITY CLASSIFICATION OF THIS PAGE
UNCLASSIFIED

CONTENTS



BIOTECHNICA '85 1ST INTERNATIONAL CONGRESS FOR BIOTECHNOLOGY, HANNOVER, WEST GERMANY

1 INTRODUCTION

The First International Congress and Exhibition, Biotechnica '85, which took place from 8 through 10 October 1985 at the Hannover Exhibition Grounds, was designed to provide an insight into the structure and growth potential of the relatively new biotechnology market. The congress, which included exhibitions, seminars, workshops, and panel discussions, focused on the commercial and industrial applications of biotechnology. The congress received support from the European Community because of its emphasis on fostering contacts between industry and academia. Over 170 companies, as well as research institutes and universities from 15 countries, disseminated information about current developments in research, laboratory equipment, and production techniques.

The topics in the scientific program presented at the symposia sessions were divided into three general areas: (1) measurement of process control and development of models; (2) biocatalyst preparation, utilization, and improvement; and (3) animal and plant cell cultures. A detailed list of the presentations is shown in Table 1.

A US seminar on biotechnology, geared to representatives from industry, took place on 9 October, concurrent with the scientific sessions. It was sponsored jointly by the US Department of Commerce, the Foreign Commercial Service Association of Biotechnology Companies (ABC), and the Industrial Biotechnology Association (IBA). These sessions were designed to acquaint European companies with US regulations on biotechnology products and potential problems for distribution of European biotechnology products in the US. The topics covered are listed in Table 2.

There were also sessions by company representatives from the US, the UK, West Germany, and The Netherlands in which special services and equipment were described and applications discussed on the basis of questions from the audience. Some examples are:

- Industrial scale purification of monoclonal antibodies for diagnostic and therapeutic applications, Charles River Biotechnical Services, Inc. (US),
- A method for microencapsulation of living cells for large-scale production of cell proteins and *in vitro* transplantation, Danon Biotech Inc. (US and Denmark),
- Automated hollow-fiber technology for production of secreted mammalian products, Endatronics Inc. (US),
- Cellift™, a new idea for laboratory cell culture scale-up, Ventrex, (US),
- Combined action of enzyme and metal catalyst applied to the preparation of mannitol, Miles-Kali-Chemie (West Germany),
- Customer-designed affinity media, fast flow sepharose, Deutsche Pharmacia Gmb (West Germany), a branch of Pharmica, Sweden,
- Continuous on-line gas analysis and regulation of fermentation processes, Hartmann and Braun (West Germany).

Concurrent sessions concerning marketing and venture capital, entitled "Bioventure: Biotechnology Financial Forum," were geared to participants from small industrial companies as well as to those interested in founding biotechnology companies in liaison with research institutes.

There were about 2500 attendees at the Biotechnica '85 Congress, at least half of them representing technical or marketing departments in industry. The balance were research scientists from universities and research institutes already engaged in biotechnology research or interested in expanding into this area.

The following sections deal with reports of the scientific sessions at the Biotechnica '85 Congress listed in Table 1. The information presented in these sessions consisted, in part, of a

Table 1

Symposia Sessions, Biotechnica '85, Hannover, West Germany

Measurement Process Control Development of Models

Position Paper on Bioprocess Modeling and Control, H.L. Lim, School of Chemical Engineering, Purdue University, West Lafayette, Indiana.

The Recent State and Future Developments on Biosensors, M. Aizawa, Institute of Materials Science, University of Tsukuha, Ibaraki, Japan.

Principals of Modeling, J.A. Roels, Gist Brocades, R&D, Delft, The Netherlands.

Adaptive Control Principles and Applications of Biotechnical Processes, A Munack, University of Hannover, West Germany.

Process Models and their Evaluation, K.H. Bellgardt, Institute for Biotechnological Research (GBF), Stockheim-Braunschweig, West Germany.

Control Strategies for Biological Processes, G. Stephanopoulos, California Institute of Technology, Pasadena, California.

Computer Applications in the Biotechnological Industry, E.A. Falch, Fermentation Technology R&D, NOVO Industrie A/S, Bagsvaerd, Denmark.

Some Topics on Computer-Aided Operation of Biochemical Reaction Processes, S. Shioya and T. Takamatsu, Department of Chemical Engineering, Kyoto University, Japan.

Computer Based Management of Biotechnical Processes, A. Holmberg, Rintekno Oy, Espoo, Finland.

Biocatalysts--Preparation, Utilization, and Improvement

Recovery of Biologically Active Proteins, M.R. Kula, Institute for Biotechnological Research (GFB), Stockheim-Braunschweig, West Germany.

Protein Enrichment and Purification. M. Hoare, Department of Chemical and Biochemical Engineering, University College, London, UK.

Affinity Chromatography, G.T. Tsao, Purdue University, West Lafayette, Indiana.

Enzyme and Cell Immobilization, J. Klein, Institute for Biotechnological Research (GBF), Stockheim-Braunschweig, West Germany.

Biosynthesis by Enzymes, C. Wandrey, Institute for Biotechnology of the Nuclear Research Center, Jülich, West Germany.

Enzyme Catalyzed Processes in Organic Solvents, A.M. Klibanov, Department of Applied Biological Sciences, Massachusetts Institute of Technology, Cambridge, Massachusetts.

Immobilized Biocatalyst Technology for Peptide Antibiotic Production, E.J. Vandamme, Laboratory of General and Industrial Microbiology, State University of Ghent, Belgium.

Enzyme Engineering by Site-Restricted Mutagenesis, G. Winter, Medical Research Council (MRC), Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, UK.

Ethanol Production by Immobilized Cells, H. Maeda (Fermentation Research Institute, Agency of Industrial Sciences and Technology, Irabolu, Japan) and G. Oda, Research Association for Petroleum Alternatives Development (RAPAD) Tokyo, Japan.

Animal and Plant Cell Cultures

Animal Cell Culture Including Monoclonal Antibody Production. R.E. Spier, University of Surrey, UK.

New Methods in Animal Breeding: Implication for Agriculture, G. Stanzinger, ETH Zürich, Institute for Animal Production and Animal Breeding, Zürich, Switzerland.

Table 1 (Cont'd)

An Animal Cell Culture System for Production of Biologicals, A.L. van Wezel, Rijksinstitut for Animal Research, Bilthoven, The Netherlands.
Production of Human Interferon by Recombinant Mouse Cells, H. Hauser, Institute for Biotechnological Research (GBF), Stockheim-Braunschweig, West Germany.
The Industrial Production of Monoclonal Antibodies in Cell Culture, J.R. Birch, Celltech Ltd., Slough, UK.
Plant Molecular Biology: Implications for Agriculture. G. Röbbelen, University of Göttingen, West Germany.
Transfer and Regulation of Expression of Chimeric Genes in Plants, H. Kaulen, J. Schell, and F. Kreuzaler, University of Cologne, West Germany.
Biotransformation, J. Stöckigt, University of Munich, West Germany.
Plant Cell Cultures, M.W. Fowler, Wolfson Institute of Biotechnology, University of Sheffield, UK.

Table 2

US Seminar in Biotechnology

Chairman: Dr. Robert Yuan, First Secretary (Biological Sciences), American Embassy, London, UK.

1. FDA Regulatory Concerns about Biotechnology-Derived Drugs and Biologies, Dr. Henry I. Miller, Food and Drug Administrator (FDA).
   a. Investigational new drugs/biologics; safety-issues-oncogends, RNA/DNA, virus contamination; master cell bunks, quality control.
2. US Industry Concerns about FDA Regulation of Biotechnology-Derived Drugs and Biologics, Dr. Bruce F. Mackler, Association of Biotechnology Companies (ABC).
   a. Lack of Agency Scientific Depth; R&D considerations; regulatory strategies; small company perspectives; product liability.
3. FDA Regulatory Concerns about Monoclonal Antibody *In Vitro* Diagnostic Devices, Dr. Henry L. Miller (FDA).
   a. Quality control; safety and effectiveness; premarket approval, patent concern.
4. US Industry Concerns about FDA Regulation of Monoclonal Based *In Vitro* Diagnostic Devices, Dr. Bruce F. Mackler (ABA) and Dr. Henry Miller (FDA).
5. How Foreign Firms Should Interact with FDA in Developing Biotechnology Products, Dr. Bruce F. Mackler (ABC) and Dr. Henry I. Miller (FDA).

review of a particular area and was geared to newcomers to the field of biotechnology. However, recent research dealing with specific problems that have been solved or are in the process of being resolved were also presented. Reports on topics such as enzyme and cell immobilization, enzyme engineering by site-directed mutagenesis, downstream processing of biologically active proteins, and the use of animal cell cultures in the production of biologicals reflected the top level research being carried out by biotechnologists with the bulk of the presentations being given by representatives from European laboratories.

2 MEASUREMENT--PROCESS CONTROL--DEVELOPMENT OF MODELS--COMPUTER APPLICATION

The presentations in this all-day program were directed towards participants who are new to the biotechnology

field, and the presentations were, therefore, general in scope. The importance of bioprocess modeling and control; improvement of biosensors; and computer-aided operation of biochemical reaction processes, and the difficulty of developing mathematical models for biotechnical processes, were reviewed in these sessions.

Bioprocess Modeling and Control

In an excellent review, H.C. Lim (School of Chemical Engineering, Purdue University, West Lafayette, Indiana) pointed out that the numerous reactions and the complexity of self-regulation of living cells make the task of modeling bioreactors extremely difficult and challenging. Knowledge of the many metabolic control mechanisms and metabolic responses to intra- and extracellular environment allows the manipulation of the environmental factors to force the cell to function to the advantage of the biotechnologist. The environmental factors that can be controlled include pH, temperature, ionic strength, dissolved oxygen, growth rate, substrate nutrients, growth factors, and trace elements. Lim stated that because it is impossible and mathematically intractable to model the living cell in all its intricacies, one should consider only those aspects of cellular behavior needed for the application of the model.

Included among the numerous reasons for wanting to model a bioreactor are: (1) bioreactor development and design; (2) bioreactor control; (3) bioreactor optimization; (4) basic and fundamental understanding of the dynamic behavior of cells; (5) simulation in place of expensive and time-consuming experimental work; (6) potential tools for testing hypotheses concerning cellular mechanisms; and (7) guideline information needed to develop more effective strains, particularly through recombinant DNA technology. For the last three purposes, a model of considerable complexity and sophistication is required. One approach would be to develop a structured segregated model from the population-balance point of view; another would be to develop a structured non-segregated model for a single cell that incorporates major pathways and regulation, then apply a finite representation technique in which the behavior of the population is represented by a finite number of single computer cells. The population-balance models have predictive capabilities of considerable significance, but the resulting equations are difficult to solve. The single-cell model requires extensive knowledge of regulation of growth, metabolism, and product formation. Lim suggested that one can begin with a crude model with adjustable parameters which is updated frequently, using responses caused by perturbations of environmental factors; optimization is then repeated in the updated model.

Reactors containing recombinant cells with plasmid instability result in mixed cultures of plasmid-containing and plasmid-free cells. Lim stated that modeling of mixed cultures of recombinant cells will receive more attention in the future. Mammalian and plant tissue culture technology is developing rapidly, yet there are very little modeling efforts in this area. Models can play particularly important roles in assessing quantitative trends and circumventing the need to carry out time-consuming and expensive experiments.

Controls of bioreactors can be broadly classified into two types: (1) dynamic (set-point) control, in which the parameters are changed dynamically, and (2) regulator control, in which the parameters are maintained at desired constant values. In batch and batch-fed fermentation, the state of the system changes dynamically, and it is anticipated that the environmental factors need to be varied optionally. For continuous reactors, the environmental factors need to be controlled at constant but unknown optimum values. The optimal profiles and optimal constant values may change due to variations in the feed and adaptation by the organisms. Thus, there are two types of problems: determination of

optimal operation conditions and frequent reassignment of these conditions. On-line learning schemes can play an important and essential role in the absence of an adequate model. The idea is to learn about the bioreactor from the responses caused by intentional perturbation, then implement an optimal control based on this information. This type of on-line adaptive control may be used to determine quickly the optimal continuous bioreactor operation conditions and to keep track of the optimum as changes take place.

Kinetic models of growth and product formation can be used quickly to determine *a priori* whether a batch-fed culture can be advantageous. At least one nonmonotonic rate expression is necessary. When no adequate models are available, batch and semi-batch bioreactors may be optimized on-line while using an on-line adaptive control scheme or off-line using the result of one run to optimize the next run. Modeling, optimization, and control must be interactive. Many bioreactor parameters are either difficult or impossible to measure on-line. On-line measurement of these parameters is required to realize feedback control. Thus, there is an urgent need to develop sensors for on-line measurements. In the absence of sensors, schemes are needed to estimate those parameters which cannot be monitored on-line.

With the availability of extensive computing facilities at relatively modest cost, it is expected that emphasis will be increased in the areas of sophisticated structured models, optimization, and control of bioreactors. Integration of molecular models of plasmid replication, segregation kinetics, and host-plasmid interaction into a sophisticated single-cell model may some day provide guidelines to improve industrial strains by recombinant technology. However, development of adequate models for organization and control is expected to be unrealistically time-consuming and expensive. Therefore, adaptive optimization and control along with on-line estimation schemes appear to be a solution for the immediate future. When adequate models become available, they can be incorporated into the adaptive control system.

The Recent State and Future Developments in Biosensors

M. Aizawa (Institute of Materials Science, University of Trukuba, Ibaraki, Japan) presented a concise report on biosensors. Advances in sensor technology have opened the way for new and sophisticated measurement techniques and instrumentation for biotechnological process control. A biosensor can offer high selectivity for a specific substance; the new breed of biosensors presents a unique marriage of immobilized biochemicals, transducers, and microelectronic components to allow almost instantaneous determination of substrate, analyte, or ligand concentration.

Matrix-bound enzyme has been amalgamated with the following electronic devices to form an enzyme sensor: (1) electrochemical devices--amperometric and potentiometric enzyme sensors, (2) thermistor--enzyme thermistors, (3) ion sensitive field effect transistors (ISFET)--Enzyme FET, (4) metal oxide semiconductor field effect transistor (MOSFET), (5) surface acoustic wave devices (SAW), (6) photodiode--enzyme photodiode, and (7) fiber optic devices.

Electrochemical enzyme sensors (electrode type of enzyme sensor) have found promising applications, particularly in clinical and process measurements. Many bench-top analyzers equipped with an enzyme sensor are commercially available, mostly for the measurement of serum components. For example the concentration of the serum components can be read out within 10 to 60 seconds after it is injected onto a matrix-bound enzyme. The analyzer can be automatically flushed and calibrated at the push of a button. Recently such instruments have been modified for use in process measurement.

Matrix-bound microbial whole cells have been used successfully to make biosensors for acetate, ethanol, glutamate,

and ammonia gas. An analyser with an auto sample has been used for on-line measurement of glutamate in a glutamate fermentation process. However, further development of sensor materials is required to improve the long-term stability and durability of these biosensors.

Many novel enzyme sensors have been proposed during the past few years. An enzyme FET, in which an enzyme layer is fixed on the gate insulator, has gained keen interest in the field of electronics; this indicates a possible crossover of biotechnology and electronics, namely "bioelectronics." Not only single, but multi-enzyme FET's have been fabricated on a single chip. Thus, semiconductor process technology has been applied to biochemical processing.

An optical biosensor, a device to generate an optical signal due to molecular recognition, offers new possibilities relative to electrochemical biosensors. The most exciting possibility offered by optical biosensors is the use of multiwave lengths and temporal information. Aizawa has recently developed an enzyme photodiode which involves light-emitting enzymes on top of the photodiode. Another type of optical biosensor is a fiber-optic-based biosensor which incorporates an enzyme phase on the end of an optical fiber. Although the research and development of optical biosensors is only beginning, a wide variety of approaches and systems are certain to emerge in the next few years according to Aizawa.

Single enzymes, however, represent only one class of biological materials that can be used for molecular recognition in a biosensor. There have been several attempts to construct an immunosensor, ranging from the measurement of transmembrane potential of a bound antibody, to a shift in potential of antibody-bound electrodes. In attempts to construct an immunosensor based on enzyme-linked immunoassay, the principles of not only electrochemical but bioluminescence, electrochemical luminescence, and fluorescence are being explored.

Aizawa also reported on a new type of a bioaffinity sensor using the biotin-avidin system which is being studied in his laboratory. This bioaffinity sensor may be applied in general to determine the concentrations of hormones and pharmaceutical drugs which are apt to lose their activities by the usual labeling with macromolecular biocatalysts. Although research into immunosensors and bioaffinity sensors is still in its early stage, these sophisticated sensors have enormous potential due to the sensitivity which can be achieved.

Computer Applications in the Biotechnical Industry

E.A. Falch (Fermentation Technology R&D, NOVO Industrie A/S, Bagsvaerd, Denmark) gave a comprehensive discussion of the use of computers in biotechnology in industry. Numerous methods for the application of computers for monitoring and controlling of biotechnological processes have been published in the last 20 years. However, during most of this period, little was known about whether these methods were actually applied in industry. This situation has changed recently. Since 1981, several reports have been published which clearly demonstrate that computers are basic elements of most contemporary control systems. The acceptance of computers in the biotechnical industry has been supported by the significant progress which has been made during the last few years in several fields: system configuration, hardware sensors, programing language, control models, and algorithms.

The early computer-based systems applied supervisory control schemes, and the systems were backed by analog controllers and hard-wired sequence controllers. A modern system uses direct digital control and is composed of several computers in a hierarchy. The tasks and the process units are distributed on control computers which can perform most functions independently. A high degree of redundancy is built into the system both with respect to computers and communication links. Such a configuration fulfills the demand for reliability, which is the desired demand

for an industrial control system. The hardware has improved with respect to speed and memory sizes. Today, both the computer and the peripherals are constructed to perform reliably in a plant environment. In addition, the flexibility of each component has been extended.

A new generation of control languages has replaced the machine-code programing of the early systems and the high-level languages of the 1970's. The programing is done through fill-in-the-blanks procedures in a general sequence language covering all aspects of process control. According to E.A. Falch, the programs are easily understood and applied by the plant personnel. The sequence controller, which is used for batch and continuous control as well as for calculations, is combined with program packages for recipe handling, reporting, and data collection. The programing of the complete system is done on one central computer through the use of advanced editing facilities and with the ability to load new and modified programs on-line.

There is still a great demand for the development of reliable on-line sensors for the variables of fermentation processes. The most important advance in the last decade has been the improvement in off-gas analysis. According to Falch, mass spectroscopy is probably the most valuable tool for determining the state of growth and product formation in a fermentation process. The application of mass spectrometers has increased the speed, accuracy, and stability of these measurements, which form the basis for the control of nutrient feeding and aeration/agitation.

Much work is being done now to adapt automated chemical analyzers to act as on-line process sensors. Progress has been made but industrial applications are few because of the lack of reliability under plant conditions. However, automated analysis with a response time of 10 to 30 minutes may serve as a valuable aid for control in an open-loop scheme if a suitable process model exists.

Two trends for the future of control of bioprocesses are seen. The first trend is an extension of the control of single-process units to an engineering optimization and, further, to an economic optimization of the process. A second trend is the application of computers as self-tuning controllers and expert systems to replace (or compensate for the lack of) detailed process and plant models. Both of these trends hold promise for future applications in the biotechnical industry.

S. Shioya and T. Takamatsu (Department of Chemical Engineering, Kyoto University, Japan) discussed computer-aided operation of biochemical reaction processes focusing, as an example, on the control of the specific growth rate in baker's yeast batch-fed culture. The use of computer control of fermenters has increased significantly in recent years. Computer control makes it possible to achieve advanced operation not only in plants where the economic advantages are obvious, but also in research laboratories. The computer provides fast and efficient data acquisition, ability to monitor and control experimental conditions, and flexibility in the operation of the fermenter.

Shioya and Takamatsu have developed an advanced control system which utilizes a simple mathematical model and a Proportional Integrative Derivative (PID) feedback controller; it also includes automatic parameter tuning for a baker's yeast batch-fed culture. If the sugar feed is low, the volumetric productivity of a cell mass will not become maximum. On the other hand, if there is excess sugar feed, the sugar accumulation in the medium will permit ethanol production even in the presence of sufficient oxygen. The ultimate goal of the control system is to maintain the ethanol concentration at a constant value in order to attain maximum volumetric productivity and cell yield. Since cell concentration increases markedly during the cultivation process, the problem of parameter adjustment of a PID controller with such an increase along with a sudden change in feeding

conditions (such as pump capacity and glucose concentration) must be considered when constructing a successful control system for fed-batch cultures. The developed control system is a combination of the experimental feeding and the PID control of the ethanol concentration. The parameter of the PID controller should be changed following the increase in cell concentration. Finally, the parameter of the PID controller is tuned automatically based on the given function of cell concentration in the feeding medium.

The results obtained from the system described above were extended to a practical and useful computer scheme so that a key parameter would follow as accurately as possible a desired profile specified in advance. For example, growth rate can be followed by the control scheme not only at a maximum constant value but also at a given arbitrary profile. A control scheme called Program-controller/Feedback-compensator (PF) system was proposed. This control system consists of a programmed controller which should follow the desired profile unless there is noise or disturbance, and a feedback compensator which should compensate for the disturbance and the noise. Proposed as the feedback compensator was the model reference adaptive control (MRAC) algorithms. The PF system with MRAC was named PF-MRAC in which the second term, MRAC, means that the MRAC was used for the feedback compensator. A classical proportional integrative (PI) feedback controller was also adapted as the feedback compensator; the entire control scheme with PI was named PF-PI. PF-MRAC and PF-PI were applied to the profile control of the specific growth rate. Numerous computer simulations using an experimentally identified mathematical model verified the usefulness of the proposed control system. PF-MRAC was found to be better than PF-PI for controlling the error of the specific growth rate when it deviated from the desired profile. Finally, the proposed profile control of the specific growth rate was realized experimentally in a baker's yeast batch-fed culture. For this, the specific growth rate itself must be observed or estimated. Low cell concentrations can be measured by a UV-spectrophotometer (used by these researchers in their experiment). The specific growth rate can be estimated using the observed cell concentration. For high cell concentrations, there is no practically available sensor for cell concentration without sampling and diluting. Another technique (for example, the macroscopic balance based on the off-gas analysis) was used for the on-line estimation of the overall growth rate. Using the rate, the specific growth rate was estimated by the extended Kalman filter. The experiments were performed using a microcomputer coupled with a laboratory-scale fermenter. The results showed that the estimation and control schemes were sufficiently useful for profile control of the specific growth rate.

3 BIOCATALYST--PREPARATION, UTILIZATION, AND IMPROVEMENT

Recovery of Biologically Active Proteins

M.R. Kula (Institute for Biotechnological Research [GBF], Stockheim-Braunschweig, West Germany) discussed the problem of the recovery of biologically active proteins. She and her group have been involved in research in this area for the past few years and have recently developed an aqueous two-phase system for the extractive recovery of intracellular enzymes and other biologically active proteins. The method has high potential and is already used industrially (see *ESN* 40-2:39-43 [1986]).

Proteins are linear polymers composed of 20 different amino acids. The exact and unique sequence of the constituent amino acids determines the folding of the polypeptide chain to a more or less globular form with a defined surface: the sequence also determines the specific interaction of such subunits to a functional entity, which may contain several identical or nonidentical polypeptide chains. The physiological or catalytic activity of a protein depends

on the integrity of such complex structures because the specific interactions with receptors or substrates occur in molecular dimensions. This complexity of protein structure has two consequences: (1) the biosynthetic machinery of cells is needed for the industrial production of proteins because a chemical synthesis lacks the necessary precision and economy and (2) the native structure of biologically active proteins must under all circumstances be preserved (or restored) during isolation and purification. Preservation of protein structure makes the task of separating one desired protein from a complex mixture quite difficult and limits applicable environmental conditions and exposure time.

The required degree of purification for a given protein will depend on its ultimate application. Technical catalysts require only certain enrichment and the absence of few interpreting enzymatic activities. Enzymes employed for analytical or genetic engineering purposes require a considerably higher purity. The highest specifications have to be met with proteins for therapeutic uses, especially those which are administered intravenously.

The general scheme followed in the downstream processing of proteins is shown in Figure 1. The methods used will depend on the scale of operation. In the laboratory, methods of protein purification are developed to a high degree of sophistication and perfection, but there are considerable gaps in biochemical engineering studies applied to unit operations involved and problems encountered in the processing of large industrial amounts.

In general, the isolation of a single protein from a complex mixture of essentially similar molecules requires more than one step. In order to achieve high yields the number of steps has to be reduced and efficiency of the single steps improved. Modern developments have led to an integration of process steps. Advances in membrane application, liquid-liquid extraction, continuous processing, and automation of various unit operations are fields of active research and increasing importance in downstream processing of biologically active proteins.

A list of some recent publications by Kula et al., and others using various approaches to purification and recovery of biologically active proteins in genes is given in the reference section.

Protein Enrichment and Purification

M. Hoare, Department of Chemical and Biochemical Engineering, University College, London, UK, presented a review of the many problems which must be dealt

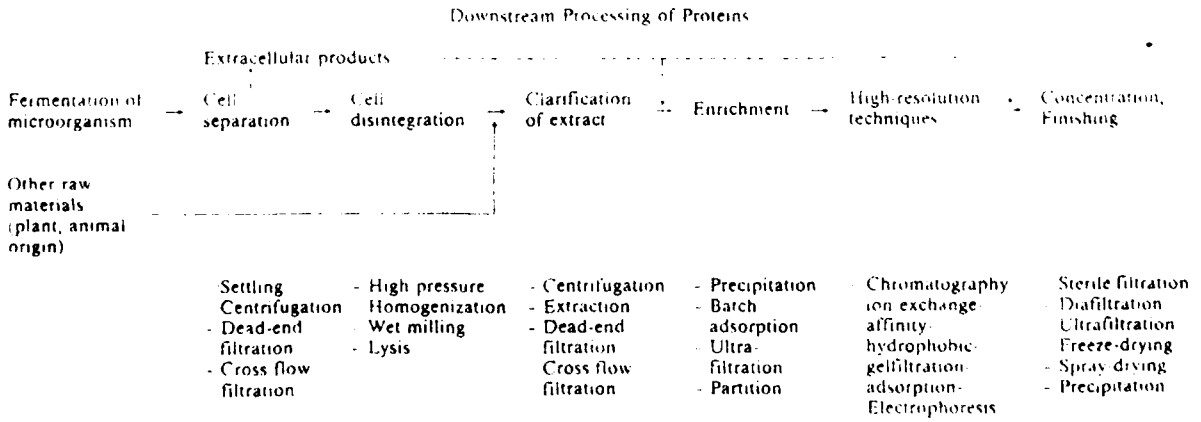


Figure 1. Downstream processing of proteins.

with in obtaining enrichment and purification of protein. Present-day industrial processes for the recovery and purification of microbial proteins and enzymes are generally based on a carefully integrated sequence of unit operations. A common theme for both extracellular and intracellular products (after removal of cells or cell debris components) is the enrichment and purification of a desired protein from a complex mixture of proteins and other soluble biological materials. Such enrichment and purification is often achieved in two broad stages: (1) a low-cost, high throughput stage where the limitation is generally the final protein purity which may be achieved; and (2) achieving the final desired protein purity where, especially for therapeutic proteins, it is necessary to employ high-cost stages (for example, affinity chromatography, gel filtration, etc.).

The design of the first stage of a protein purification process will necessarily need to take into account the conditions adopted for fermentation and fermentation broth harvesting. The reason is that minor variations in broth composition can have a considerable effect on the success of the downstream processing. Reliable operations are required with performance characteristics which do not fluctuate too widely with variations in the process stream composition. One such operation is that of protein precipitation and recovery (developed by M. Hoare and his group). Others are the use of two-phase aqueous liquid-liquid extraction (developed by Kula et al.) or ion exchange technology. Also, under investigation is the incorporation of product and cell manipulation into the process design aimed at aiding downstream processing operations--advantage can be gained by adopting genetic engineering techniques.

Protein Precipitation and Recovery. The design and operation of a protein precipitation and precipitate recovery process involves analysis of a number of operating parameters: (1) the formation of precipitate nuclei; (2) precipitate growth and aging; and, finally, (3) precipitate transfer to a high-speed centrifuge, and recovery and dewatering therein. From an economic viewpoint, it must also be taken into account that the desired protein may appear in the soluble or precipitated phase and that the centrifuge stage will account for most of the capital expenditures in the precipitation process. The main design questions that must be considered are: (1) how to prevent loss of yield due to protein denaturation, (2) how to effect a high degree of resolution between precipitated and soluble proteins, and (3) how to efficiently recover the precipitate from the soluble phase with good resolution and with the centrifuge operating at a high throughput. These problems have been addressed by Hoare and others and at this time, the protein precipitation method appears to be a worthwhile procedure in protein enrichment and purification.

Affinity Chromatography

For protein purification G.T. Tsao, Purdue University, West Lafayette, Indiana, discussed affinity chromatography, a separation technique in which a substance is selectively adsorbed from solution into an insoluble adsorbent through interactions with a particular functional group or a whole molecule immobilized on the adsorbent. In principle, affinity chromatography makes it possible to separate protein mixtures into their components on the basis of their ability to recognize and bind a specific structure. The basis for separation is markedly different from the conventional methods used with proteins which rely on differences in gross physical properties such as molecular weight, solubility, and isoelectric point. Examples of the affinity chromatography method commonly utilized are the binding specificity between: (1) an enzyme and its substrate, substrate analogs, inhibitor, or cofactors; (2) an antigen and its antibody; and (3) a hormone and its receptor.

Although extensive experimental work has been published on the use of adsorption chromatography, very little

theoretical work has been done on affinity chromatography, according to G.T. Tsao. Tsao stated that this is due to the fact that even though the theoretical aspects of chromatography in general have been investigated at length, the majority of them are based on the assumption of either linear equilibrium isotherm or linear or bilinear adsorption kinetics. Therefore, the theoretical aspects are not readily applicable to the analysis of affinity chromatographic systems for which a nonlinear equilibrium isotherm of Langmuir type is usually assumed.

Recently, a modified plate model (equilibrium-stage model) of chromatography with sufficient sophistication has been developed to satisfactorily account for the nonlinearity of the equilibrium isotherm. The most vigorous way to model a system is to formulate and solve a set of differential equations. However, in many cases, this approach is very complicated, and a new approach is needed. The plate model for the nonlinear chromatographic system is an example of this. The modeling is accomplished by first identifying the problem and then trying to adjust the proper term in the available analytical solution of the corresponding linear systems. In short, the concept of local or stagewise linearization is used. With the plate model, a complicated system like the nonlinear affinity chromatographic process has been analyzed successfully for the first time.

Enzyme and Cell Immobilization

A review of this subject was presented by J. Klein, Director, Institute for Biotechnological Research (GBF), Stockheim-Braunschweig, West Germany. Enzymes and whole cells are immobilized for the following reasons: (1) to solve the problem of catalyst retention, thus allowing for the reuse of the catalyst in discontinuous and continuous processes; (2) to obtain high concentrations of the catalytic species as a basis for a significant reduction of the reactor volume; and (3) to stabilize (considerably) the catalytic function provided by an enzyme or cell.

In considering enzymes and whole cells as biocatalysts there is no clear distinction with regard to the respective fields of application. It is quite clear that enzymes (for example, hydrolases and esterases) are primarily used in one-step reactions. Coimmobilization of purified enzymes plus coenzymes has only been studied on a small scale. With cells, the whole range from one-step reactions (preferably with dead cells) to complex multienzyme reactions (including viable resting or growing cells) can be covered wherever the alternative "enzyme or whole cell" exists. It will be simply a question of economics as to which method to chose.

Methods developed for immobilizing enzymes and cells include: adsorption, cross-binding, covalent attachment, entrapment, and microencapsulation. The carrier materials used are organic and inorganic with a strong preference for synthetic and natural organic polymers. Enzymes are preferably immobilized by covalent binding on a preformed, well designed carrier to ensure optimum efficiency during immobilization and application. Entrapment methods are primarily used for whole cells since less pore-size control is required for a complete cell retention in a matrix. For industrial application of immobilized enzymes and cells, adsorption and cross-linking techniques are also being considered. Only limited information is available in the literature about those immobilized biocatalyst processes which are used industrially. Most catalyst development for industrial use is done "in house," which means that in many instances information about industrial catalysts is not published in the scientific literature. With the intention of improving the comparison and evaluation of immobilized biocatalysts also on a technical scale, "Guidelines and recommendations for the characterization of immobilized biocatalysts" has been developed and published by the European Federation of Biotechnology (EFB) working party on "Applied Biocatalysts."

With regard to large-scale applications some well established processes can be mentioned such as: (1) immobilized enzymes: glucose isomerization and cleavage of penicillin and (2) immobilized cells: L-aspartic acid, L-malic acid, L-alanine. Others, like the production of ethanol with immobilized living cells, have been established on an industrial pilot scale. Klein believes that more emphasis will be given to cofactor-dependent multienzyme reactions and, therefore, more developments will be forthcoming in the area of viable immobilized cells. Such viable cells can be used as resting cells when the product formation is decoupled from cell growth. Operational lifetime of such catalysts, based on cell growth by reincubation with the carrier matrix, provides means for an extended use in a repeated reaction/regeneration mode. Other reactions, like ethanol fermentation are only feasible under steady-state but limited growth conditions. Recent findings that surface-attached yeast cells have a significantly higher productivity than suspended cells are a strong argument for the usefulness of immobilized growing cells. Another new aspect for an improved design of immobilized enzyme and cell systems is the extended use of organic solvent in biocatalytic reactions.

Biosynthesis by Enzymes

This topic was discussed by C. Wandrey, Institute for Biotechnology of the Nuclear Research Center, Jülich, West Germany. Until now the degrading and transforming capabilities of biocatalysts have been used more intensively in biotechnology than their synthetic potential. This is due to the fact that energy-rich precursors are needed for biosynthesis, which normally can only be prepared economically in a living cell. Additionally, there is the "coenzyme problem"--redox-equivalents can only be passed from one reactant to the next by means of transport metabolites (coenzymes). In order to re-use these expensive "shuttle molecules" it is not logical to immobilize the enzymes because they would be unable to fulfill their transport task. Both problems--to obtain energy-rich precursors and to use coenzymes continuously--have been solved in several instances, at least in the laboratory.

Acetyl phosphate, which can be synthesized classically, can be used as an energy source to regenerate ATP from ADP. By this means, it is possible to synthesize a peptide from non-protected amino acids using specific enzymes. Wandrey and his research group have been able to produce γ-glutamyl-cysteine continuously in a membrane reactor by means of an ATP-regenerating system which was driven by the energy-rich acetyl phosphate. Such a system may be regarded as a classical example for biosynthesis because these enzymes are all synthetases.

Oxido-reductases may also be regarded as enzymes for biosynthesis, at least if they are used for the generation of chiral centers. The products obtained this way are often called chiral syntons. A typical example is the production of a non-natural L-amino acid (L-tertiary leucine) from the corresponding α-keto acid by means of stereospecific reductive amination. This process was developed by Wandrey and his group in collaboration with Kula (Institute for Biotechnological Research, GBF, Braunschweig, West Germany) and Degussa AG, Hanau (Dr. Leuchtenberger). The precursor (α-keto acid) is synthesized classically while the transformation into the L-amino acid is achieved in an enzyme reactor. This time, the driving energy source is formate, which is decarboxylated to yield hydrogen by means of an isolated enzyme (formate dehydrogenase).

The most common energy source of an entire microorganism is glucose. Up to now, an effective use of this energy source has only been possible by means of glycolysis in the entire cell. For biosynthesis, one is more interested in using glucose as an energy source in a series of enzymatic steps than as a carbon source for promoting growth of the entire microorganism. In accordance

with this concept, the growth of an entire cell should only be promoted as long as it is logical to increase the concentration of the catalyst. After this, it is possible to keep the concentration of the cells (catalyst) constant by means of the limitation of some other nutrients. Now, a series of enzymatic reactions starting from a classically synthesized precussor can be driven with glucose as an energy source. Using this route, it is possible to continuously produce branched chain L-amino acids like L-leucine.

In addition to oxido-reductases, transferases, and ligases, hydrolases can also be used for biosynthesis. The best known example of a reverse reaction of a hydrolase is the use of a peptidase to produce peptides. One route to obtain aspartam, a peptide sweetener widely used today, takes advantage of this principle. Wandrey believes that in the future, the use of organic solvents will play a more important role so that it may become possible also to transform or even synthesize more lipophilic products. Wandrey also stated that for the future, the greatest potential for biosynthesis by enzymes will be done via protein engineering. The production of insulin and human growth hormone by means of *E. coli* shows that it is possible to program a microorganism to produce a natural but not strain-specific compound. Thus it appears possible to design enzymes which will be able to produce non-natural compounds in a microorganism.

Enzyme Catalyzed Processes in Organic Solvents

A.M. Klibanov, Department of Applied Biological Sciences, Massachusetts Institute of Technology, Cambridge, Massachusetts, spoke about the recent use of organic solvents in enzyme-catalyzed processes in biotechnology. In the past, the commonly assumed necessity of conducting enzymatic reactions in aqueous solutions had eliminated the study of enzymes as catalysts in organic chemistry. Klibanov and his research group have recently found that many different enzymes (hydrolases and oxido-reductases) can act vigorously as catalysts in a number of nearly anhydrous organic solvents. Such enzymatic reactions obey Michaelis-Menton kinetics. The dependence of the catalytic activity of enzymes in organic media on the pH of the aqueous solution from which they were recovered is bell-shaped with the maximum coinciding with the pH optimum of the enzymatic activity in water. The catalytic power exhibited by enzymes in organic solvents is comparable with that displayed in water. When transferred from water to a non-aqueous environment, enzymes become remarkably thermostable according to Klibanov.

Some enzymatic processes can be carried out on a practical scale only in organic solvents because these processes are nearly impossible in water due to kinetic and thermodynamic reasons. Preparatively important examples of the above, developed in the laboratory of Klibanov, include: (1) production of optically active compounds via lipase-catalyzed stereospecific esterifications and transesterifications, (2) lipase-catalyzed regioselective acylation of glycols, and (3) polyphenol oxidase-catalysed regiospecific hydroxylation of phenols.

Immobilized Biocatalyst Technology for Peptide Antibiotic Production

This topic was reviewed by E.J. Vandamme, Laboratory of General and Industrial Microbiology, State University of Ghent, Belgium. Immobilized biocatalyst technology for producing known or new antibiotics is gaining much interest. This concept is being applied to the field of peptide antibiotic bio-conversion and fermentation. The use of immobilized enzymes, organelles, and cells to synthesize antibiotics as an alternative to conventional fermentation is already in progress. *In vitro* total enzymatic antibiotic synthesis, for example, is illustrated with the "multi-enzyme thio-template mechanism" for the synthesis of gramacidin C, enniatin, etc. Total synthesis of peptide antibiotics based on immobilized living

cells has recently been demonstrated with penicillin, bacitracin, nisin, nikkomysim, and with a few other non-peptide antibiotic compounds such as tylosine and cyclosporin A.

In industry, enzymes or cells are already being used to convert peptide antibiotics into therapeutically useful derivatives, free and immobilized penicillin acylases producing the penicillin nucleus, and 6-amino-penicillanic acid (6-APA). Their potential for synthesizing semi-synthetic β-lactam antibiotics (penicillin, cephalosporin, nocardicins, and monolactams) is under intensive investigation by industry. Microbial acylases, acetylesterases, and α-amino ester hydrolases, acting on cephalosporin compounds and yielding valuable intermediary compounds and end products, have also gained wide interest.

Stereo-specific enzymic side chain preparation for production of semi-synthetic penicillin and cephalosporin has recently reached the industrial stage. Bioconversion possibilities with the novel peptide β-lactam compounds are also being studied actively.

These examples of simple-step (bioconversion) as well as complex multistep enzyme reactions (fermentation) point to the vast potential of immobilized biocatalyst technology in fermentation science, in organic synthesis, and in biotechnological processes in general.

Enzyme Engineering

G. Winter, Medical Research Council, Laboratory of Molecular Biology, Cambridge, UK, discussed the application of recombinant DNA technology for the purpose of engineering of enzymes and other proteins, such as antibodies. These proteins can now be altered at the level of the gene. This permits structure-function studies of the roles of individual residues in the catalytic mechanism, substrate binding, and large molecular interactions. For example, in the enzyme tyrosyl tRNA synthetase, mutation of various surface lysine and argenine residues has allowed the mapping of the path of the tRNA across the surface of the synthetase. However, in addition to such academic applications, this technology may permit the tailoring of enzymes for industrial or medical uses. It has already been possible to construct an enzyme with improved affinity for substrate and an improved catalytic rate, to design an oxidation resistant protease inhibitor, and to join an enzyme to the variable domain of an antibody. Winter and Fersht (1984) have published a detailed review of this topic (see reference section).

Ethanol Production by Immobilized Cells

H. Maeda, Fermentation Research Institute, agency of Industrial Sciences and Technology, Ibaraki, Japan, and G. Oda, Research Association for Petroleum Alternatives Development (RAPAD), Tokyo, Japan, presented some of their recent work on ethanol production. The batchwise fermentation process for ethanol fermentation still predominates today. However, in order to produce fuel alcohol more economically, it is necessary to attain much improved productivity and to reduce manufacturing costs in comparison with the conventional process. For these requirements, several processes such as a yeast cell recycling system and a flocculated cell system have been proposed. RAPAD, organized under the auspices of the Ministry of International Trade and Industry of the Japanese government, decided to develop two kinds of continuous fermentation processes using immobilized growing yeast cells. Both are based on the recent developments in immobilized cell technology. One is the process using immobilized yeast in a gel calcium alginate. The other is the process using immobilized yeast in a gel containing a photo-crosslinkable resin. The basic research has already been done by Maeda and Oda, and research on the pilot plant scale is now being carried out. For large-scale production, the following must be satisfied: (1) good conversion yield by prevention of contamination, (2) prolonged viability of the immobilized carrier, and (3)

practical operability during the fermentation steps.

Maeda and Oda have used the yeast *Saccharomyces cerevisiae* for the process equipped with immobilized yeast cells entrapped by calcium alginate gel. The continuous preparation of immobilized cell beads was carried out by showering drops of sodium alginate solution containing live yeast cells into calcium chloride solution in the bioreactors. The content of sodium alginate in the mixture was finally adjusted to 3 to 4 percent. The preparation of cell beads was completed within several hours. No special equipment was needed for the preparation of the beads. As a result of studies of the prevention of microbial contamination, it was found that contamination could be effectively prevented when the initial pH of the inlet substrate solution was kept at 4.0 with sulfuric acid. The addition of some bactericidal substances was also found to be effective. Thus, the process was operable without sterilization of the inlet medium.

The process flow diagram of the pilot plant operation is in Figure 2.

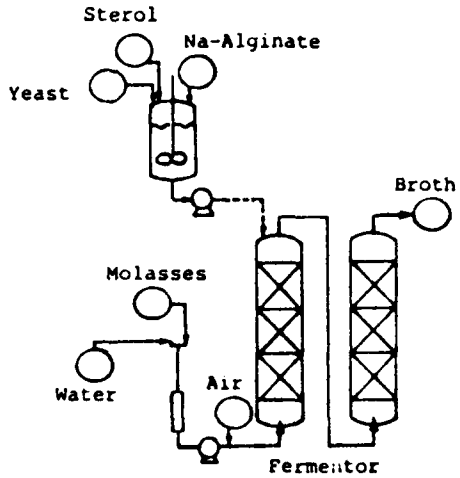


Figure 2. Process flow for the pilot plant operation.

This pilot plant is composed of two reactor channels. One channel consists of two columns (each 1 kL) in series and the other consists of three columns (0.8 kL for one and 0.6 kL for the other two). The total column volume is 4 kL and the total productivity is 2.4 kL ethanol/day, thus 600 L of pure ethanol can be produced each day by using a 1-kL column reactor. As a result of the pilot operation, 8.5 to 9.0 percent (v/v) ethanol was produced constantly from diluted cane molasses for over 4000 hours (about 6 months).

Saccharomyces species was used as the yeast for the process equipped with immobilized yeast cells entrapped by a photo-crosslinkable resin. The photo-crosslinkable resin was about 310 Å long and the chain polymer was composed of 65 percent polyethlene glycol moeity and 35 percent polypropylene glycol moiety. The structure of photo-crosslinkable resin is shown in Figure 3.

The immobilized yeast gel was prepared as follows. The mixture of 40 g photo-crosslinkable resin, 60 g yeast suspension, and a very small amount of benzoyl peroxide was spread on a plastic sheet and exposed to light at wave lengths of 300 to 400 nm to produce a yeast sheet of 0.8 to 1.0 mm thickness. The yeast sheet was placed parallel to the flow direction of the substrate solution. The process flow diagram of continuous ethanol fermentation of the pilot plant is shown in Figure 4.

The record of the pilot plant operation covering about 3000 hours showed that the immobilized yeast used

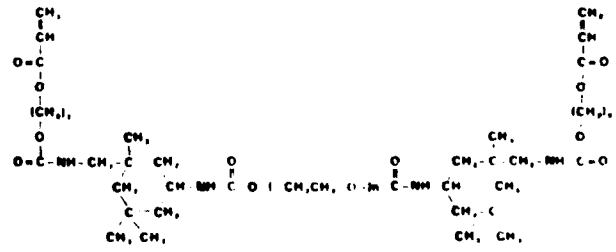


Figure 3. Structure of photo-crosslinkable resin.

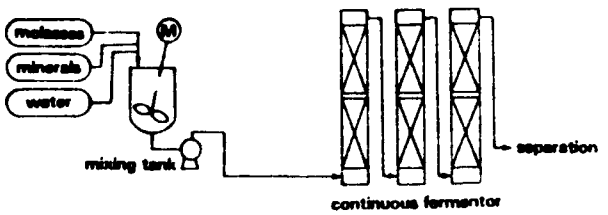


Figure 4. Process flow for continuous ethanol fermentation.

in this process was able to maintain stable activity for a long period and that contamination was almost completely controlled by a very low concentration of sodium metabisulfite under non-sterilized fermentation. The cell concentration, final ethanol concentration, yield of sugar, fermentation time, and productivity were 40 g/L, 8.5 g/100 ml, 95 percent yield, 5 h, and 11 kg ethanol/m^3 h, respectively.

4 ANIMAL AND PLANT CELL CULTURES

Animal Cell Culture Including Monoclonal Antibody (Mab) Production

R.E. Spier, Department of Microbiology, University of Surrey, UK, presented an excellent overview of the present status of the use of animal cell cultures in biotechnology. These cultures had been regarded as difficult to achieve and expensive to produce for the following reasons: (1) animal cells are fragile and break down when exposed to the technologies of the fermenter or other processing equipment; (2) it is difficult, if not impossible, to run large scale cultures of animal cells free from exogenous contamination; (3) the cost of the serum which is necessary for the growth medium for animal cells prohibits the more general exploitation of such systems; (4) the lack of a chemically defined medium means that it is difficult to reproduce the effects achieved in one run on the next, supposedly identical, run; and (5) materials produced from animal cells in cultures may be carcinogenic.

While most of the contentions outlined above seemed to be important in the 1960's and even into the 1970's, the evidence today shows that these problems no longer exist. It is now relatively commonplace for animal cell cultures to be run at scales of 8000 liters in stirred tank reactions. Such systems are in use for the production of α-lymphoblastoid interferon from Namalwa cells and for the production of foot-and-mouth disease vaccines. There is also an active program for the development of air-tight fermenter systems for the production and exploitation of hybridoma cell lines producing monoclonal antibodies (Mabs) at the 1000-liter scale. This development could lead to similar types of reactors operating at scales of 10,000 liters or more, according to Dr. Spier. For each of the systems mentioned above, the cells used in the large reactors have been "hardened" to the environment of the bioreactor by a program of selection and phenotypic adaptation and weaning. Such activities in the stringent environment of the commercial organization demonstrate clearly that the contention that animal cells are delicate, fragile entities does not always apply.

Less demanding cell adaptation processes have been used to obtain cell cultures which require solid surfaces for their growth. The technology for such systems has been transformed radically during the past 10 years. Whereas the traditional system of choices for scale-up has been to increase the multiplicity of units (normally static or rolling bottles), the availability of a number of alternative technologies has made it practicable to design and run larger sized unit process systems. Such systems are now commonly used at the 1000-liter scale for the production of polio vaccine from VERO cells grown on the surface of microcarriers held in suspension in a stirred tank reactor. There have been recent reports that in Japan β-fibroblastic interferon is being produced in a similar system at scales of between 5000 to 20,000 liters.

Such large systems would be uneconomic if they required the use of fetal calf serum (costing about $110 to $140

US per liter) to a concentration of up to 10 percent of the medium. It has been found possible to use instead, alternative sera which are much less expensive. For example, adult bovine serum can cost as little as $5 to $15 per liter with newborn calf serum being somewhat more expensive but still much less than fetal calf serum. An additional feature of the use of adult sera is that the probability of a viralogical or mycoplasmic contamination due to improperly collected or sterilized serum is decreased considerably. Serum-containing media are not a necessary prerequisite for fully transformed cells. Monoclonal antibody-producing cells are made from either the fusion of a fully transformed cell with an untransformed antibody-producing cell or they can be formed by the Epstein-Barr virus-caused transformation of an antibody-producing cell. In both cases, it is possible to grow such cells in media which are not supplemented with whole animal serum but which are made up from a definable basal medium containing relatively simple and generally chemically defined supplements such as insulin, transferrin, selenium, and certain unsaturated free fatty acids.

It is not unusual for the technology which has been used for the cultivation of animal cells to be somewhat ahead of its counterpart technology in other areas of microbial biotechnology. The use of a microprocessor in Spier's laboratory for the interactive set-point control of analog computers may be cited as a case in point. It also seems that present developments in the way in which concentrated cell systems are handled in the animal cell area may prove to be of interest to microbial biotechnologists in other areas. While there are many examples of the use of gels to provide a matrix for the immobilization of a wide range of cell types (yeast and algae as well as animal cells) there are relatively few reports of the use of trapped cell systems from areas other than those of the animal cell biotechnologists. Such trapped cell systems have been used for the production of Mabs. At present, there is fierce competition between the proponents of microsphere entrapment systems and those who advance the alternatives of entrapment in the "shell side" of a capillary bundle or between the decks of planar membranes held in a stacked or cassette form.

The technological problems for the cultivation of animal cells have been essentially surmounted and continue to provide opportunities for new concepts and developments. Media problems are also succumbing to systematic efforts at arriving at an inexpensive and reliable fluid in which to grow animal cells. There is also progress on the way in which the products of animal cells are viewed by the regulatory agencies. Understanding of the mechanism of cell transformation and the relations of this phenomenon with oncogenes, retroviruses, and growth factors and their receptors should in the future lead to an increase in our ability to use animal cells for the benefit of all.

An Animal Cell Culture System for the Production of Biologicals

This topic was discussed by A.V. van Wezel, Rijksinstitute For Animal Research, Bilthoven, The Netherlands. At the beginning of the 1960's the concept of Unit Process for production of bacterial vaccines was introduced by Dr. van Hemert at this institute. It was based on the application of the homogenous culture technique as generally applied in microbiology. The basic principle of this concept implies that for the production of various vaccines the same standardized equipment for cultivation and downstream processing is used. This approach has the advantage that on the basis of time sharing, the same production facilities and manpower can be used for the production of different vaccines. Through the development of the microcarrier culture techniques, this concept is being extended to the cultivation of animal cells for the production of viral vaccines.

Presently, the knowledge and experience built up in the field of animal cell cultivation is being applied to the

production of biologicals such as Mabs, plasminogen activator, and other biologicals cloned in animal cells by recombinant DNA techniques. van Wezel and his group have recently developed a continuous perfusion cultivation system for the large-scale production of these products. In such a system, medium is continuously added to the bioreactor with a rotating filter fixed on the stirrer shaft, and the culture fluid removed. In this way, high cell densities and product yields can be obtained. The system described above lends itself very well for continuously operating processes and for large scale production of animal cell biologicals. The perfusion cultivation system can be prepared in relatively small bioreactors in conjunction with satellite equipment for medium preparation and product concentration as a first step in the downstream processing.

Production of Interferon by Recombinant Mouse Cells

H. Hauser, Institute for Biotechnological Research (GBF), Braunschweig, West Germany, discussed some of the problems involved in the application of recombinant DNA (rDNA) technology to the production of biological products. He presented some of the research work from his group. Until recently, the use of rDNA for the production of biological products has been primarily directed towards expression of cloned DNA in prokaryotes. However, the bacterial environment is not suitable for proper expression and modification of many eukaryotic gene products such as glycoproteins like Mabs, interferons, and vaccines. For the development of an economic process in mammalian cells, the specific genetic manipulation as well as the engineering problems of the cells have to be solved. In an ideal process, a stable cell line, easy to handle and inexpensive to maintain, should steadily secrete the desired product into a medium from which it can be purified in a few steps.

Human fibroblast interferon (huIFNβ) is a glycoprotein (N-glycosylation) secreted transiently by a variety of cells in response to virus and induction protocols including double stranded RNA (dsRNA), inhibitors for protein, and RNA synthesis. The huIFNβ is not only able to confer an antiviral state on its target cells, but also displays a wide range of biological properties, like antiproliferative and antitumor activities. Induction results in a transient expression of the messenger RNA (mRNA) that has a comparatively short half-life. The huIFNβ peptide has been shown to be produced by conventional methods upon engineering of its DNA in bacteria and yeast as well as in insect and vertebrate cells.

Interleukin-2 (IL-2) is a 15,000-dalton glycoprotein (O-glycosylation) transiently secreted by T-cells upon stimulation with antigen or plant lectins. Apart from its well known activity exerted on T-cell growth, several additional activities have recently been ascribed to this factor, including Ig-synthesis-promoting activity on B cells. For the most part, such studies have been conducted by using nonglycosylated IL-2 from *E. coli*, poorly characterized factor preparations, or IL-2 differing in post-translational modification from the naturally occurring IL-2 molecule.

Although theoretically any permanent animal cell line can be transformed with DNA, effective genetic engineering is dependent on several conditions such as high transformation rate, promoter control, integration of the transgenome, etc. The cell line must be able to correctly process the gene product and keep the transferred DNA stable without selection pressure. Conditions for high expression of a given gene upon transfer in a selected cell line are due to the chromosomal state and location, the copy number, the transcription from the promoter, and the translation of the mRNA as well as to the respective half-life time for mRNA and protein product.

Hauser presented his research on the expression of huIFNβ and IL-2 in several cell lines upon transfer of the

chromosomal genes under the control of different promotors. He found that the use of viral promoters for expression in mouse L cells gave the best results with both genes. Due to the chromosomal site of integration of the transgenome and the number of integrated functional copies, the transformed cell clones exhibited variability in expression of the products. Houser stated that because it is presently impossible to achieve high expression by locating the transgenome in a specific way, screening of single cell clones with respect to high expression is essential.

Selected high producer mouse L cell clones were characterized with respect to product authenticity and to biotechnological use. Human IFNβ from mouse L cells was characterized and found to be indistinguishable from authentic huIFNβ with respect to antiviral activity on different cell lines, competition and immunoprecipitation by specific antibody, and to specific antiviral activity. The molecular weight is identical with IFNβ from human FS-4 cells, indicating a similar, if not identical glycosylation. Further analysis, however, is required to study the glycosylation pattern of the murine product. The investigations are being carried out.

Mouse L cells have been used for fermentation to produce human glycosylated IFNβ as an alternative to the production in primary human foreskin cells. Several properties indicate that this cell line is advantageous for production: (1) the cells populate a microcarrier fermentation unit in a short time period; (2) a selected clone is genetically stable and keeps producing huIFNβ permanently for at least 6 months on the same microcarrier batch and (3) huIFNβ is secreted into the medium even at low serum concentrations, leading to a high specific activity for starting the purification procedure.

IL-2 from normal human blood lymphocytes was prepared by Hauser et al. They showed that the purified factor consists of several glycosylated forms and an additional nonglycosylated component. The peptide backbone of all forms appeared to be identical. The IL-2 from mouse 2 cells shows a similar pattern of glycosylation and is active with respect to T-cell growth activation.

The Industrial Production of Monoclonal Antibodies (Mabs) in Cell Culture

J.R. Birch, Celltech Ltd., Slough, UK, discussed this topic, emphasizing the work at Celltech Ltd. The development of the hybridoma technique by Köhler and Milstein in 1975 for the production of Mabs led rapidly to the exploitation of these reagents as highly specific molecular probes in many areas of research. Since then the commercial interest has developed rapidly and many products based on Mabs are being marketed, particularly in medical diagnosis. Besides *in vitro* use there is increasing interest in the development of *in vivo* applications for Mabs, both as diagnostic probes (for example, in tumor detection and as therapeutic agents *per se*). In addition to these medical applications, antibodies are being used as immunopurification reagents. Many of these applications require the production of hundreds of grams to kilogram quantities of antibody per year. The early production methods based on growing hybridoma cells as ascites tumors in rodents are inappropriate at this scale. Therefore, alternative manufacturing processes based on cell culture *in vitro* have been sought. Numerous cell culture methods have been proposed for the large-scale production of Mabs. Broadly, one can distinguish between homogeneous suspension culture and perfusion systems in which cells are entrapped or immobilized in semi-permeable hollow fibers, microcapsules, microbeads or ceramic cartridges. Celltech Ltd. decided on the former because it offered the most straightforward and predictable route to scale up, according to Birch.

The particular type of reactor which the company has used for hybridoma culture is the airlift fermenter. The principal advantages of this type of fermenter are (1) simplicity (no moving parts such as motors and stirer shafts), (2) good mixing, and (3) good mass

transfer characteristics. It was found that the airlift reactor has very effective oxygen transfer characteristics, unlike many cell culture systems which are incapable of supplying sufficient oxygen at high cell densities (greater than 10^6 cells/ml). Efficient oxygen transfer is maintained as scale-up increases (Birch et al., 1985). Both the oxygen requirement of hybridoma cells (typically about 7 $\mu g/10^6$ cells/hr) and the effect of dissolved oxygen concentration on growth and metabolism were both measured.

Airlift fermenters of 5-, 10-, 30-, 100-, and 1000-liter working volumes have been operated both in development laboratories and in the company's production facility. More than 30 cell lines of mouse, rat, and human origin, all producing Mabs, have been grown in these fermenters. More than half the antibody produced is synthesized during the decline phase of growth. Product yields vary from 40 to 500 mg/liter with an average for all cell types of 105 mg/liter.

In most of the work to date, culture media supplemented with animal sera has been used. However, Celltech™ has now developed a serum-free medium suitable for use in airlift fermenters. This medium has several advantages, including lower cost and reduced protein concentration resulting in easier recovery of antibody. The production process is based on batch culture principles. Birch and his group, however, are examining continuous chemostat culture, both as a tool for process optimization and as a potential production method. They are now operating a pilot system (30 liter) based on an airlift fermenter to examine the process engineering problems involved in scale-up. In addition, they have been operating with biomass feedback in order to increase the productivity of the culture. With a mouse hybridoma producing an IgM antibody, an output of 380 mg/day from a 5-liter airlift chemostat with feedback has been achieved. This represents a 5.4-fold increase compared with the output from a chemostat without feedback. The antibody must be removed from the culture medium after the fermentation stage. Thus, cells are separated by continuous flow centrifugation and the culture supernatant concentrated by tangential flow ultrafiltration. Depending on the characteristics and intended use of the antibody, subsequent purification involves a combination of various precipitation, ion-exchange chromatography, and affinity chromatography steps.

5 CONCLUSION

The Hannover Biotechnology Conference (BioTechnica '85) focused attention on the transfer of research results to industrial application with the aim of encouraging more of this transfer. Thus, besides the scientific sessions, there were special sessions geared towards fostering interaction between universities, research institutes and industry. In addition, the exhibition of products and technologies was more extensive than at previous biotechnology congresses held in Europe during this year. The presentations in the scientific program were excellent and showed that biotechnology research in Europe and the UK is of high caliber and represents a greatly increased emphasis on basic as well as applied research in biotechnology.

6 REFERENCES

Bell, D.J., M. Hoare, and P. Dunnill, "The Formation of Protein Precipitates and Their Centrifugal Recovery," *Advanced Biochemistry Engineering Biotechnology*, 27 (1984), 1-7.

Birch, J.R., K. Lambert, R. Boraston, P.W. Thompson, S. Garland, and A.C. Kenney, "The Industrial Production of Monoclonal Antibodies in Culture," in *NATO Advanced Studies Institute Symposium Series* (Plenum Press, New York: 1985) in press.

Fish, M.N. and M.D. Lilly, "The Interaction Between Fermentation and Protein Recovery," *Biotechnology*, 2 (1984), 623-627.

Flaschel, E., Ch. Wandrey, and M.R. Kula, "Ultrafiltration for the

Separation of Biocatalysts," *Advanced Biochemistry Engineering Biotechnology*, 26 (1983), 73-142.

Hustedt, H., K.H. Droner, U. Menge, and M.R. Kula, "Protein Recovery Using Two-Phase Systems, *Trends in Biotechnology*, (1985), 1-7.

Janson, J.C. and P. Hedsman, "Large-Scale Chromatography of Proteins," *Advanced Biochemistry Engineering*, 25 (1982), 43-99.

Kula, M.R., *Recovery Operations in Biotechnology*, ed. H.J. Rehrm and G. Reed (VCH Verlags Geseelshaft, 2 1985), 726-760.

Kula, M.R., K.H. Droner, and H. Hrestedt, "Purification of Enzymes by Liquid-Liquid Extraction," *Advanced Biochemistry Engineering Biotechnology*, 26 (1982), 73-118.

Recktenwald, A., K.A. Kruner, and M.R. Kula, "On-Line Monitoring of Enzymes in Downstream Processing by Flow Injection Analysis (FIA)," *Enzyme Microbiology Technology*, (1985), in press.

Winter, G. and A.R. Fersht, "Engineering Enzymes," *Trends in Biotechnology*, 2 (1984), 115-119.

END

DTIC

FILMED

4-86