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Abstract

The author visited the conference: "BIO Technica' 85" in Hannover, 8-10 october 1985. This report summarizes some of the experience from the part of the conference that dealt with measurement, modelling and process control.

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Uppskattningsvis var 600-900 personer samlade till en tredagars mässa, konferens och seminarier. Cirka 170 företag var representerade. Till konferensen var talare inbjudna för översiktspresentationer. De tre dagarna var koncentrerade till följande temma: I) Mätteknik, modellbygge och reglering. II) Biokatalysatorer - preparation, användning och förbättringar. III) Djur och växtcellodlingar, genteknik. Där fanns också en del seminarier kring finansiering och ekonomi som pågick parallellt. Avsikten är att starta en serie med årliga bioteknik mässor i Hannover. EG står bakom och stöttar ekonomiskt. Nästa mässa är 23-25/9 1986.

Nedan intryck jag fått med speciell tonvikt på det som intresserar oss och speciellt mig. I appendix finns konferensprogram samt abstrakt till några föredrag.

Korta intryck från själva mässan

Det formligen drällde av mikrodatorer och persondatorer kopplade till analysinstrument och demonstrationsprocesser. Det var IBM-PC, Apple och Digital som man såg mest. Där fanns en hel del tillämpningar av grafik. Det mest spektakulära var representationen av en antibiotikamolekyl. Den kunde vändas och vridas och man kunde dels få se en streckfigur, och dels molekylen med 'van der Waals-molnen' och då var atomer som ligger bakom varandra skymda. Indata var resultat från röntgenundersökningar. Beräkningarna var gjorda på stor dator och all data var överförd till persondator för presentation.

Etanolgivaren hade en grupp i Braunschweig arbetat vidare på. Stommen var fortfarande en Figaro sensor men bärgasen var borta. Givaren var mer linjär över intressant mätområde. Företaget INGOLD i Schweiz kommer att sälja den.

Instrument för samtidig cellräkning, storleksbestämning, fluoroscensmätning utvecklas vidare. Fluorocensdelen innebär att molekyler inne i cellen, befintliga eller speciella märkmolekyler, exciteras med laser, och emitterat ljus ger mått på cellinnehållet. Till exempel kan DNA och RNA innehållet i populationen följas. Pris ca 200 000 kr.

Mätteknik baserad på ultraljud och Doppler shift fanns representerad och även något byggt på opto-akustisk teknik. Ett av föredragen tog upp potentialen i optiska biosensorer. Man tänkte sig enzymer eller antikroppar i ändan på optiska fibrer. Med laserteknik kan förändringar på optiska fiberns ända följas. Se [Aizawa, Tsukuba].

Rintekno

Jag pratade en del med Andrea Holmberg och hon visade deras data system för processreglering. Ett sådant system har installerats på Jästbolaget och Andrea har speciellt varit involverad i detta. Detta system bygger på PDP11/73. (Man har också enklare system för IBM-PC.) En hel del arbete var nedlagt på smidig hantering och presentation av loggade data. Färggrafik användes. Vidare var det förberett för att lätt kunna koda sin egen regulator och stoppa in i systemet. Koden skall skrivas i FORTRAN av något slag.

Programvaran är utvecklad i samarbete med konsultföretag. På vilket sett man löst realtidsproblemen framgick inte. Jag träffade också Aulis Rajala, Manager, section Pharmaceuticals and Biochemicals. Han berättade om ett utvecklingsprojekt med Pharmacia. Rintekno expanderar.

Tips till vårt jästprojekt

Shioya såg fedbatchreglerproblemet så att man under första halvan styr på tillväxthastigheten för att få högt 'Meissel-tal', vilket är relaterat till RBC (ratio of budding cells). Senare delen styr man på att hålla etanolkonc nere. För att reglera tillväxthastigheten använde han någon form av Kalmanfilter utgående från mätningar av OD (optical density). Vid låga celltätheter Fungerar OD mätningar bra. Etanol signal användes inte i detta Kalmanfilter (!). Resultat från körning visade att tillväxthastigheten höll sig på börvärdet, men styrsignalen visade våldsamma fluktuationer. Shioya erkände att han inte var riktigt nöjd med detta, när jag frågade. MRAS hade använts här. Se artikel i: Chem. Eng. Vol. 40, No. 3, 499-507, 1985.

Bellgardt pratade jag lite med, men fick inte ut så mycket. Han talar mycket i black-box termer och har (får) för lite fysikalisk/biologisk insikt. Se [Bellgardt]. Han arbetade numera med modellbygge av process med animalieceller i en hollow-fiber reaktor. Han kommer till IFAC.

Någon i Purdue-gruppen med prof. H.C. Lim tittar också på Fiechters data på jäst. Detta var bara något som sas i förbifarten i hans föredrag, då han talade om multiple-steady state i CSTR. För övrigt ett bra föredrag. Se [Lim].

Etanol produktion

Där var en man, dr Maeda, som berättade om pilot anläggningen i Japan för kontinuerlig etanol produktion med immobiserad jäst. Speciella studier hade gjorts av immobiliseringstekniken. Två metoder var patenterade (?). Den ena var i Ca-alginat likt vår medtod och den andara benämndes 'photo-cross linking'. Vidare var det viktigt att tillföra lite syre för cellens maintenance funktion och det var också viktigt att tillföra steroler. Se nedan under Stephanopoulus. Några siffror: residence time 5 h (fluidized bed); packing ratio 33.7%; pH=5.3%; Socker in 20 g/dℓ melass (? jag är osäker); Cellerna håller 6 månader. Se [Maeda, Tsukuba].

Träffade i förbifarten en ung engelsman, dr Mitchell, som doktorerat på etanolproduktion med immobiliserad jäst. Nu jobbade han i industrin. Han nämnde problemen med gasutvecklingen. Gasflödet var enligt honom ca 10 ggr större än vätskeflödet. Jag uppfattade det hela som ett scale-up problem.

Många jobbar istället med en bakterie Zyomona Mobilis. Den har högre utbyte men i gengäld är den sämre i andra avseende. I Hannover var det en ung doktorand i reglerteknik som påbörjat modellbygge och diskuterade det vid en av montrarna. Clemens Poster heter han. Han menade att glukos till etanol var enkelt modellerat, men det var mycket viktigt med inverkan av extra näringsmedel samt biprodukter vid jäsningen. Vidare var inhibering pga hög etanolkonc viktigt men svårt att modellera. Han hade viss biologisk insikt men det var inte testat tillräckligt mot labdata. Han kommer till IFAC i december.

Där var en stor paneldiskussion anordnat av 'Landwirtschaftliche Arbeitsgruppe Bioethanol und Biogas, Bonn'. Ekonomin diskuterades och man talade om spannmålsöverskottet i EG och om metanol, etanol och bilmotorer in på 90-talet.

Intressanta reglerproblem - Stephanopoulos synpunkter i synnerhet

Lim från Purdue University i USA höll ett intressant föredrag om modellbygge och reglering av bioprocesser. Han talade speciellt om: processer med multipla jämviktslägen; karaktäristik hos fedbatch profiler baserade på tillväxt- och produktbildning som funktion av substratkoncentration; reglering av processer där organismer med plasmider ingår. Vidare berättade han kort om optimering av en bioprocess, baserad på gradientmedtod. Se [Lim].

Stephanopoulos menade att där finns en del reglerproblem som folk tiger om för att man inte får det hela att fungera. Hans föredrag byggde på mer på erfarenheter från informella samtal än på det som är publicerat. Typiskt tog han upp exempel på multipel steady-state i CSTR. Det är i några generella fall intressant att ligga kring en instabil jämviktspunkt. Ett exempel är odling av 'recombinant E. coli' där avvägningen gäller tillväxt gentemot produktbildning. Vanlig PID duger ej. Vidare är där exemepel på oscillationer i syreupptagnings-förmåga inducerade av skift i utspädningshastighet - Zyomona Mobilis. Oscillationer i tillväxten har också observerats för 'methylothrophs', (SCP) då de växer på methanol. Jag uppfattade något som liknar icke minfas beteende. Butanol processen innehåller också knepig dynamik. I vissa lägen produceras Acetoin istället för Butandiol. Grupp på ETH kring Dunn jobbar med detta. Till sist berättade Stephanopoulus om vikten av syresättning till jäst vid etanol produktion. Syrenivå skall ligga kring 20-30 ppb. Ej mätbart. Hur reglera detta? Etanolproduktiviteten går upp drastiskt, stationärt sett, vid rätt syrenivå. Sterol innehållet i cellmembranet påverkar börvärdet på DO. Statiska experiment hade gjorts i CSTR och cellkoncentration hade mätts som funktion av syresättning vid konstant vätske flöde. Hysteres i denna kurva hade iakttagits och vidare var vidden på hysteresen en funktion av sterolkonc. Just kring hysterespunkten var etanolproduktiviteten som högst. Se [Stephanopoulos].

Nya möjligheter på det molekylära planet

Det talades om möjligheterna att göra egna enzymer. Bland annat hade man lyckats att göra ett enzym som binder till tRNA. En stor potential ser man i förståelsen av hur antigener bildas. Eventuellt tänker man sig utnyttja antigenens flexibla struktur för att göra egna katalysatorer. Se [Winter].

Ett område som man jobbar på, på MIT, är möjligheten att låta enzymer arbeta i organiskt lösningsmedel istället för vatten. Lösningsmedlet påverkar starkt i vilken konformation ett enzym antar och bestämmer därmed i hög grad dess funktion. En upptäckt man gjort är, att det räcker med ett monolayer av vatten molekyler runt enzymet för att det ska fungera. Resultaten gäller enzym som bryter ner lignin. Ett dussintal enzym har fåtts att funger i organiska lösningsmedel. Lignin är en stor outnyttjad råvara, om jag förstod det hela rätt. Se [Klibanov].

8th – 10th October, 1985
1st International Congress + Exhibition for Biotechnology

BIO Technica'85 Hannover

Conference

Abstracts

CONGRESS PROGRAM

Tuesday, 08.10.85

I. MEASUREMENT – PROCESS CONTROL – DEVELOPMENT OF MODELS – COMPUTER APPLICATION

			Abstract No.
09.30-10.15 a.m.	Position paper on bio process modelling and control	Lim, West Lafayette, Indiana/USA	1
10.30-11.00 a.m.	a) The present state and future development on biosensors	Aizawa, Tsukuba/J	2
11.00-11.30 a.m.	b) Principles of modelling	Roels, Delft/NL	3
11.30-12.00 a.m.	c) Adaptive control principles and applications to biotechnical processes	Munack, Hannover/D	4
12.00-12.30 p.m.	d) Process models and their evaluation	Bellgardt, Stöckheim/D	5
02.00-03.00 p.m.	Control strategies for biological processes	Stephanopoulos, Pasadena/USA	6
03.30-04.00 p.m.	a) Computer application in bio industry	Falch, Bagsvaerd/DK	7
04.00-04.30 p.m.	b) Some topics on computer-aided operation of biochemical reaction processes	Shioya, Kyoto/J	8
04.30-05.00 p.m.	c) Computer based management of biotechnical processes	Holmberg, Espoo/SF	9

SESSION CHAIRMAN: in the morning : Prof. Thoma, Hannover/D
in the afternoon : Prof. Schügerl, Hannover/D

Wednesday, 09.10.85

II. BIOCATALYSTS – PREPARATION, UTILIZATION AND IMPROVEMENT

09.15-10.00 a.m.	Down stream processing of biological active proteins	Kula, Stöckheim/D	10
10.30-11.00 a.m.	a) Protein enrichment and purification	Hoare, London/GB	11
11.00-11.30 a.m.	b) Affinity chromatography	Tsao, Lafayette/USA	12
11.30-12.00 a.m.	c) Enzyme and cell immobilization	Klein, Stöckheim/D	13
12.00-12.30 p.m.	d) Biosynthesis by enzymes	Wandrey, Jülich/D	14
02.00-03.00 p.m.	Enzyme-catalyzed processes in organic solvents	Klibanov, MIT/USA	15
03.30-04.00 p.m.	a) Immobilized biocatalyst technology for peptide antibiotic production	Vandamme, Ghent/B	16
04.00-04.30 p.m.	b) Enzyme engineering by site-directed mutagenesis	Winter, Cambridge/GB	17
04.30-05.00 p.m.	c) Ethanol production by immobilized cells	Maeda, Tsukuba/J	18

SESSION CHAIRMAN: in the morning : Prof. Chmiel, Stuttgart/D
in the afternoon : Dr. Kula, Braunschweig/D

BIO Technica'85 Hannover

Thursday, 10.10.85

III. ANIMAL AND PLANT CELL CULTURES

09.15-10.00 a.m.	Animal cell culture including monoclonal antibody production	Spier, Guildford/GB	19
10.30-11.00 a.m.	a) New methods in animal breeding implications to agriculture	Stranzinger, Zürich/CH	20
11.00-11.30 a.m.	b) Animal cell culture for production of biologicals	van Wezel, Bilthoven/NL	21
11.30-12.00 a.m.	c) Production of human interferon by recombinant mouse cells	Hauser, Stöckheim/D	22
12.00-12.30 p.m.	d) Industrial production of monoclonal antibodies in cell culture	Birch, Slough/GB	23
02.00-03.00 p.m.	Plant molecular biology implications to agriculture	Röbbelen, Göttingen/D	24
03.30-04.00 p.m.	a) Transfer and regulation of expression of chimeric genes in plants	Kreuzaler, Köln/D	25
04.00-04.30 p.m.	b) Biotransformations	Stöckigt, München/D	26
04.30-05.00 p.m.	c) Plant cell cultures	Fowler, Sheffield/GB	27

SESSION CHAIRMAN: in the morning : Prof. Schmidt-Kastner, Wuppertal/D
in the afternoon : Prof. Hartmann, Braunschweig/D

ABSTRACT OF

POSITION PAPER ON BIOPROCESS MODELLING AND CONTROL

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A living cell is an expanding biochemical reactor in which perhaps thousand(s) of biocatalytic reactions take place under a highly sophisticated "master" control system which has evolved through eons of adaptation and survival. It is this built-in metabolic regulation system which provides the cell the ability to alter its biocatalytic reactions to a changing environment in some optimal fashion. These thousand(s) of reactions and the complexity of self-regulation make the task of modeling bioreactors extremely difficult and challenging. Knowledge of numerous metabolic control mechanisms and metabolic responses to intra- and extra-cellular environment would allow us to purposely manipulate the environmental factors to force the cell to function to our advantage. The extracellular environmental factors one can control includes pH, temperature, ionic strength, dissolved oxygen, growth rate, substrate nutrients, growth factors, and trace elements.

Since it is impossible and mathematically intractable to model the living cell in all intricacies, it is important to consider the ultimate purpose(s) of modeling and consider only those aspects of cellular behavior needed for the application of the model. Once various purposes of modeling are examined then one can look at complexity and level of sophistication required for individual purpose. There are numerous reasons for wanting to model a bioreactor, some of which are i) for bioreactor development and design, ii) for bioreactor control, iii) for bioreactor optimization, iv) to obtain basic and fundamental understanding of dynamic behavior of cells, v) for simulation purpose in place of expensive and time consuming experimental work, and vi) for use as potential tools for testing hypotheses concerning cellular mechanisms and provide guideline information needed to develop more effective strains particularly through recombinant 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 and the other would be to develop a structured nonsegregated model for a single cell incorporating major pathways and regulation and then use 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

BIO Technica'85 Hannover

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.

For preliminary design calculations and regulator type control perhaps the simplest models, unstructed and nonsegregated may suffice. However, for the purpose of optimization the model to be developed must reflect the effects of those manipulated variable with which the reactor is to be optimized, for example, the environmental variables such as pH, temperature, dissolved oxygen and chemical variables such as nutrients, growth factors, trace and elements. Although various models of varying complexity are available in the literature, invariably most of them do not reflect the effects of environmental factors. Considering that a cell contains many potentially rate limiting steps and the actual control steps depends upon the history and nature of environmental changes, it is a formidable task to model these effect. Thus, an alternate approach is suggested. One can begin with a crude model with adjustable parameters, which is then updated frequently using responses caused by perturbations of environmental factors and optimization is repeated based on the updated model.

Reactors containing recombinant cells with plasmid instability result in mixed cultures of plasmid-containing and plasmid-free cells. Modeling of mixed cultures of recombinant cells will receive more attention in the future. Mammalian and plant tissue culture technology is developing rapidly and yet there is very little modeling efforts in this area. In view of expensive media and time consuming experimentation involved, models can play particularly important roles in assessing at least qualitative trends and circumventing the need to carry out time-consuming and expensive experiments.

Controls of bioreactors can be broadly classified into two; dynamic (set point) control in which the parameters are changed dynamically and regulatory control in which the parameters are maintained at desired constant values. In batch and fed batch fermentation the state of system changes dynamically and it is anticipated that the environmental factors need to be varied optimally, while for continuous reactors the environmental factors need to be controlled at constant but unknown optimal 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 reassessment of these conditions. Once again one faces the problem of adequate models which reflect the effects of rector operating conditions. It appears that on-line learning schemes can play an important and essential role in the absence of adequate model. The idea is to learn about the bioreactor from the responses caused by intentional perturbation and then implement an optimal control based on this information. This type of on-line adaptive control

approach may be used to determine quickly the optimal continuous bioreactor operation conditions and keep track of the optimum as changes take place.

Kinetic models of growth and product formation can be used to quickly determine a priori whether a fed-batch operation 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 using an on-line adaptive control scheme or off-line using the result of one run to optimize the next run. In fact, modeling, optimization and control must be interactive.

Many bioreactor parameters are either difficult-to-or impossible-to-measure on-line. Realization of feedback control requires on-line measurement of these parameters. Thus, there is an urgent need to develop sensors for on-line measurements. In the absence of sensors, estimation schemes are needed, which allows estimation of parameters which can not be monitored on-line.

With availability of fact and extensive computing facilities with moderate cost, it is expected that more extensive efforts be forthcoming in the areas of sophisticated structured models, optimization and control of bioreactors. Integration of molecular models of plasmid replication and 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 optimization and control is expected to be unrealistically time consuming and extensive. Therefore, adaptive optimization and control along with on-line estimation schemes appear to be a solution for immediate future. When adequate models become available they can be incorporated into the adaptive control system. Application of artificial intelligence and expert system is expected to make an important contribution in the near future.

BIO Technica'85 Hannover

The Recent State and Future Developments on Biosensors

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Adavances in sensor technolgy have opened the way for new and sophisticated measurement technniques and instrumentation for biotechnological process control. Among various sensors a biosensor can offer high selectivity for a specific substance, which has not been attained by synthetic sensor materials. The new breed of biosensors will present a unique marriage of immobilized biochemical, transducer and microelectronic components to allow almost instantaneous determination of substrate, analyte or ligand concentration.[1,2)]

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 thermistor.
(3) Ion sensitive field effect transistor (ISFET): Enzyme FET.
(4) Pd MOSFET.
(5) Surface acoustic wave (SAW) devices.
(6) Photodiode: Enzyme photodiode.
(7) Fiber optic devices.

Electrochemical enzyme sensors (electrode type of enzyme sensor)

have found promissing applications in especially clinical and process measurements. Many a bench-top type of analyser equipped with an enzyme sensor are commercially available mostly for serum component measurement. The sample is injected on to matrix-bound enzyme and the concentration of serum component read out whithin 10-60 s. The instrument is automatically flushed and calibrated at the push of a button. The instrument has recently been modified for use in process measurement. A full automatic analyser for glucose, alcohol, lactate and glycerol may be supplied by a Japanese producer, although it is a bench top type.

Matrix-bound microbial whole cells have successfully been used to make biosensors for acetate, ethanol, glutamate, ammonia gas, and BOD. An analyser with an auto sampler has been used for on-line measurement of glutamate in a glutamate fermentation process.[3]

Further development of sensor materials is strongly required to improve the long term stability and durability of these biosensors.

Many novel enzyme sensors have been proposed these several years. An enzyme FET, in which an enzyme layer is fixed on the gate insulator, has gained keen interest in the field of electronics, which indicates a possible cross-over of biotechnology and electronics, namely "bioelectronics". Not only single but multi-enzyme FET's have been fabricated on a chip. It is noted that semiconductor process technology has been applied to a biochemical proessing.

An optical biosensor, which is a device to generate optical signal due to molecular recognition, can offer new possibilities relative to electrochemical biosensors. The most exciting possibility offered by optical biosensors is the use of multiwavelength and temporal information. The author proposed an enzyme photodiode

BIO Technica'85 Hannover

which involved a light emitting enzymes on the top of photodiode.[4] Another type of optical biosensor is a fiberoptic-based biosensor which involves an enzyme phase on the end of a fiber optic. Although research and development of optical biosensors is only begining, the wide variety of approaches and systems that are possible are certain to emerge in the next few years.

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 measurement of transmembrane potential of a bound antibody, to a shift in potential of antibody-bound electrodes. In attempts to construc an immunosensor based on enzyme-linked immunoassay, not only electrochemical but bioluminescence,electrochemical lumines
cence and fluorescence principles are being explored.[5] This is in the line of optical biosensors.

The author has recently reported a new concept of a bioaffinity sensor using biotin-avidin system.[6] The concept 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.

Research into immunosensors and bioaffinity sensors is still in its early stage. These phophisticated sensors,however,have enormous potential due to the sensitivity which can be achieved.

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PRINCIPLES OF MODELLING

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Why are models, particularly mathematical models, used?
The answer is that they are used to get a better grasp on reality.
Real things, even the most simple ones, are far too complex to understand in detail. Yet, especially in engineering, one has to work with complex real things and so, if one wants to explain, to describe or to make use of even comparatively simple real things, models have to be used, be it implicitely or explicitely.
The foregoing is indicative for an important principle of modelling: the complexity of reality is to be reduced in such a way that it can be handled by e.g. mathematical methods. The author has the impression, in view of much of the literature on mathematical modelling in the last decade that this seems to have been frequently overlooked. Many publications seem to add complexity and contribute little to the handling of a reality outside mathematics, computer simulations or scientific publications per se.

In contemporary chemical engineering, mathematical models are vital to the rational design, operation and optimization of chemical plants and a well developed process technology has evolved as a result of the enormous growth of particularly the fossils based chemical industry and the large amounts of research and development invested therein. Also in microbiology and biochemistry and their commercial exploitation in biotechnology, the quantitative mathematical analysis of processes has become a more and more accepted tool. It is much less developed in that sector and the feasibility of applying rigorous mathematical methods to the intracies of the living world has often been questioned.

In the engineering sciences the basic language of the description of reality is the continuum approach. It leads to the formulation of so-called macroscopic models. In these models the microscopic structure of the system i.e. the fact that the elementary units of reality are of a corpuscular nature, e.g. molecules or microorganisms, is ignored and macroscopic variables like pressure, temperature and concentration are used.

The basic tool of the continuum theory is the balance equation and it can be constructed for each extensive quantity, i.e. a quantity which is additive with respect to the parts of the system.

BIO Technica'85 Hannover

A special class of extensive quantities is then recognised to be conservative i.e. in the balance equations for these quantities a term accounting for conversion, which in addition to the one representing transport processes appears in the general case, does not occur.
Known and useful examples of conserved quantities are the amounts of chemical elements, balances for the latter quantities are of great use in the reduction of the complexity of a system's description.

Further refinement of the use of balance equations in biotechnology is obtained if so-called biochemically structured models are used. In these methods some details of the biochemical structure of the reaction patterns in microorganisms are considered.
The description of the dynamics of biotechnological processes becomes possible if kinetic models are introduced. However, a significant problem to overcome is that the complexity of reality needs to be reduced to obtain models which are useful. As has already been stated modelling of the intricacies of reality leads to models which are far too complex to be useful, certainly if useful is interpreted in the engineering sense. A strategy towards the simplifications of reality can be based on the comparison of the relaxation times of environmental changes and those of the mechanisms relevant in the description of the system's behaviour.
In this way mechanisms can be identified which does not contribute to the dynamic behaviour of the system on the time scales relevant to the application under consideration.

The most rigorous simplification of reality in the kinetic description of bioengineering systems is obtained if so-called understructured models are used. In these models the properties of the biomass present in the culture are assumed sufficiently specified by one number, the total amount of biomass present in the culture.
Although the complexity of reality is greatly reduced in these models they are of great use in quite a number of applications and are widely accepted in both industrial and academic circles.

A number of cases exist, however, in which unstructured models fail to be adequate, in that case a structured model, in which a limited number of compositional aspects of the biomass are also considered, has to be constructed.

Structured models are not as well developed as unstructured models, although quite a number of accounts concerning the subject have appeared in the literature in the last decades. It is questionable if succesful applications of such models have been developed yet. Certainly in industry such models have not become widely accepted.
This is due to at least two problems: intricacies in the theory of the construction of structured models and the lack of experimental testing of such models.
For the future developments of useful structured models there certainly are prospects; the theory seems to be well developed. Problems are associated with the experimental verification of such models; this often calls for quite sophisticated experimental methods.
Some of the considerations which are presented will be illustrated with respect to models of the fed-batch process for the large scale production of penicillin and the production of ethanol by yeast and bacteria.

BIO Technica'85 Hannover

Adaptive Control Principles and Applications to Biotechnical Processes

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Almost ten years ago, adaptive control concepts were considered more as an art than a science. Since that time, great progress has been achieved in theoretical research on these methods, and the recent development of cheap and powerful microcomputers for process control has enabled applications in many different areas. However, there is still (and certainly will remain in the future) a certain aspect of art in this control philosophy, since it is applied to plants or processes which are not entirely known and often do not meet the theoretically necessary assumptions. Just this is the situation in control of biotechnical processes, since there the behaviour of the culture cannot be modelled entirely and the adaptation of the microorganisms to changing environmental conditions may change the behaviour of the process drastically. This fact has to be realized particularly during fed-batch cultivation, which is the most important type of cultivation conditions for biochemical processes.

In applications of adaptive control one has to divide into two different concepts, one of these may be characterized by the self-tuning controller, using formal models of the process or of parts of it, and the other by the predictive control concepts, using detailed process models. Both concepts may be applied to control of biotechnical plants, if their different features are used in an adequate way each. Self-tuning controllers may be applied in a lower level in the control hierarchy, for example in substrate feeding or aeration, the latter being strongly influenced by the medium composition and e.g. anti-foam agent. There is in fact a certain number of further control loops whose system dynamics and disturbances vary in a wide range during fed-

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batch cultivations. In the presentation, some concepts of adaptive controllers will be briefly considered and the prerequisites for applications will be discussed.

However, these basic control loops have to be provided with correct set points in order to lead the whole process to an economical optimum and to take into account mutual dependencies between several control loops. For these higher-level control tasks, adaptive optimizing strategies based on detailed process models are strongly recommended. One of these principles, the so-called Open Loop Feedback Optimal (OLFO-) Control, will be discussed using the application to an SCP process performed in an airlift tower loop bioreactor. The OLFO strategy is based on the heuristical concept of separation of identification and control. The whole (batch-) fermentation interval is divided into subintervals of constant length. During each interval, an identification of the unknown or time-varying system parameters is performed, based on measurement data of the preceding interval. The course of the identified parameters is predicted for the future (if no other information is available, the actual parameter values are considered to be valid). This enables to optimize input functions for control of the process in order to minimize a given cost functional. This functional should be based on economic data, which means the cost of substrate, aeration, cooling, downstream processing, and the gross proceeds of the process products. During optimization, the complete time interval from the present time to the end of the cultivation is considered, and optimal input control functions are computed for this interval. However, only the control during the next time interval is actually used each, since after the end of this time interval, new estimates for the parameters may be computed from recent measurement data, which lead to improved control functions for the rest of the cultivation time. In this way the procedure works during the complete batch fermentation time and performs adaptations to variations of process parameters and deterministic disturbances. However, the computation of the parameter estimates and the optimal input functions may be rather time-consuming for complex processes. This results in quite long adaptation interval lengths during which the process actually is driven in open loop. Therefore the control functions are not directly applied to the process but act

BIO Technica'85 Hannover

as setpoints resp. reference functions for some low-level controllers. Thus the system may respond quickly to disturbances, and advantages of long-term planning on the upper level and of quick responses to disturbances on the lower level are combined in this hierarchical control structure.

It should be pointed out that system modeling is a strong prerequisite for application of all these ideas: In batch or fed-batch cultures, the state of the process is driven through a great variety of different combinations, in contrast to continuous cultivations, where the state is kept almost constant. Therefore, optimization cannot be based on models which are obtained by identification of coefficients in linear and formal system equations. On the contrary, the basic structure of the process and the most important nonlinearities must be known and must be incorporated in the model, in order to obtain meaningful predictions for the future, and consequently meaningful optimal control functions. - At the same time, process simulation techniques are strongly involved in the OLFO procedure, since a great number of simulation runs are needed to compute estimates of the unknown parameters and to optimize the control functions. In contrast to other process simulation applications, these simulations here are needed on line, which requires extremely fast and reliable algorithms.

The complete procedure of modeling and OLFO-control is demonstrated by means of an SCP process in an airlift tower loop reactor. A reduced model is used for the control, consisting of a quasilinear parabolic partial differential equation describing the dissolved oxygen concentration in the liquid phase of the reactor, an implicit algebraic equation for modeling the corresponding equation in the loop, and an ordinary differential equation describing the cell concentration. Results of parameter identifications using process data and results of simulated control runs will be discussed.

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Abstract

Process models and their evaluation

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The aim of mathematical optimization methods and of modern algorithms of automatic control in biotechnology is high productivity and reproducability of the processes. The kernel of all these theoretical procedures is formed by mathematical process models. One problem of model building is the large model complexity - as a consequence of the large complexity of the processes - and the lacking knowledge in wide areas of metabolism.

To fulfil all requirements of model accuracy in the most important operating points of the process, structured growth models are often required. These can describe the dynamics of metabolic regulations during transitions between different types of metabolism. For modeling of these effects a sufficient knowledge of the microorganism's growth is needed. This comprises important biochemical reactions and superimposed biological control loops.

One problem of model building is, that the extensive knowledge of the stoichiometry of metabolism is opposed by an insufficient knowledge of the regulation. But a suitable choosen structure of the entire model can ensure, that an inaccurate model of the regulation has only a minor effect on the stoichiometric model.

The development of a structured mathematical model consisting of three submodels is demonstrated for the yeast **Saccharomyces cerevisiae**. In contrast to the dynamic models of the reactor and of metabolic regulation by induction and repression , the biological model of the metabolisms stoichiometry can be formulated as a non-linear algebraic system of equations. The latter is called quasi-stationary reaction model.

The distinct division of the over-all model into almost independent submodels - the quasi-stationary reaction model and dynamic models of metabolic regulation and of the reactor - results in a good understanding and identifiability of the model. The parameters of the stationary and dynamical submodels can then be independently identified from chemostat and batch experiments.

For development, verification and quantification of the reaction model chemostat experiments are suited very well. It will be demonstrated, how the most important types of metabolism can be investigated by special experiments and how a mathematical model can be obtained, that is also valid under growth limitations by multiple substrates. The possible types of yeast metabolism are anaerobic or aerobic growth on a varity of substrates. The parameters of the reaction submodel can be identified, independently from dynamic effects, with a special procedure, which was developed for chemostat experiments. Using this method a significant reduction of the dimensionality of the parameter identification is obtained.

The proposed parameter identification procedure consists of two steps. The first step is data reduction and extraction of significant biological characteristic parameters by linear regression of specific reaction rates measured in the experiments. In the second step the real parameter identification is performed by a simple minimization of the mean square deviation of characteristic parameters obtained from the experiments and the model. Using this method, data of several experiments can be used at the same time for the identification. This ensures uniqueness of the identified parameter values. The simulation results with the model are presented for some chemostat experiments. These are anaerobic and aerobic cultures of **Saccharomyces cerevisiae** on different substrate mixtures of glucose and ethanol.

When the quasi-stationary reaction model and its growth parameters are known, the dynamic regulation model can be developed and its parameters can beidentified. For this, batch and chemostat-shift-experiments are useful, which allow a

BIO Technica'85 Hannover

directed stimulation of regulatory adaption of the metabolism. Building the reaction model, the analysis and description of rate limiting steps (master reactions) of the growth is very important. The model of metabolic regulation can be formulated in a simple manner as a system of control loops, which are active or inactive, depending on the actual operative master reaction. In these terms, many dynamic effects such as unsymmetric responses of organisms to shift-up or shift-down experiments can be explained. To show this behaviour, simulation results of a batch-shift-experiment and a fed-batch-experiment are presented, where the substrate and oxygen supply to the culture were changed several times.

An application of the model which solves the control problems of on-line cell mass and parameter estimation for a yeast cultivation, is presented finally. To serve as a basis of an Extended-Kalman-Filter the complex structured growth model was simplified. Besides the estimation of dry cell mass concentration, two strongly variable biological paramters are estimated. Using the model, specific reaction rates for substrates and products can then be calculated on-line, which give a good sight into the running process. This example demonstrates the straightforward use of the proposed model structure. Mathematical models like this can be an effective tool for process control.

CONTROL STRATEGIES FOR BIOLOGICAL PROCESSES

Gregory Stephanopoulos

ABSTRACT

Control system of biological processes can be classified in two general categories. Those primarily designed for the control of the culture environment and those aiming at the elimination of culture instabilities and process optimization. The first category includes rather standard controls currently present in all bioreactors, such as temperature, pH and dissolved oxygen controls. These controls will be reviewed along with the control policies employed and sensors involved. In the same category also included is the manipulation of the flow of substrates or other growth factor in fed-batch fermentations introduced in recent years. The application of optimal control theory in the context of optimal profiling of the feed flow rate of fed-batch fermentations as well as optimal profiling of other environmental parameters of both fermentations will be discussed.

The second category includes controls which are in general system specific and have varying objectives. The need and potential benefits from such controls will be illustrated by a variety of examples covering a broad spectrum of biotechnological applications, both traditional and new. For example, important

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fermentations involving methylotrophs for single cell protein production, Zymomonas mobilis or Saccharomyces cerevisiae for ethanol production, and others, have been observed to oscillate. The presence of these oscillations in a production environment is generally undesired and attempts to stabilize them by conventional controllers were unsuccessful. In ethanol fermentations or amino acid fermentations (our results) productivity increases of the order of 50-100% can be realized by controlling the oxygen concentration at the appropriate levels. However, long term adaptation effects prevent the use of known controls for this purpose. Another example is provided by the antagonism between growth and foreign protein expression in recombinant microorganisms. Changes by factors of 50-100 in product formation are possible by properly controlling the environment so that growth and product formation are well balanced. Other control applications involve the early detection of a fermentor failure due to culture mutation or contamination, (such failures account for 25-30% of all batch fermentations), the stabilization of mixed cultures in continuous flow fermentors for large volume applications such as ethanol production from biomass, SCP production, etc., and the optimal profiling of fed batch reactors for antibiotic fermentations.

These examples will demonstrate the many ways by which biotechnological processes can be benefited by the application of proper controls. They will also serve as vehicles for illustrating the two basic elements of the contemplated control structures, namely, (a) the ability to determine, on-line, the state of the culture and, (b) the ability to predict the response of a biological system to various imposed environmental changes. A new methodology for the on-line estimation of the state of the culture will be presented. This methodology is unique in its ability to produce very good estimates under both

steady state and transient conditions and without making use of any models for the processes of growth and product formation. The methodology makes use of available microscopic and elemental balances and estimation-filtering theories and produces a variety of additional information through the intense manipulation of all presently available on-line measurements. The interfacing of such "intelligent sensors" with system behavior predictors in an integrated control structure will also be addressed.

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Computer Applications in the Biotechnical Industry

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Numerous methods for the application of computers for monitoring and control of biotechnological processes have been published in the last 20 years. During most of this period, however, little was known about whether these methods were actually applied in industry. This situation has changed. 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, programming languages, 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 by 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 fulfils the demand to reliability which is the decisive demand to an industrial control system.

The hardware has not only improved with respect to speed and memory size. Today both the computer and the peripherals are constructed to perform very reliable 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 programming of the early systems and also the high-level languages of the 1970's. The programming is done through a fill-in-the-blanks procedure in a general sequence language covering all aspects of process control. 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 programming 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 key variables of fermentation processes. The most important advance in the last decade has been the improvement in off-gas analysis. The application of mass spectrometers has increased the speed, the accuracy, and the stability of these measurements. This is today the most valuable tool for determining the state of growth and product formation in a fermentation process. The measurement forms the basis for the control of nutrient feeding and aeration/ agitation.

Much work is being done now to adapt automated chemical analysis equipment 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 10-30 minutes may serve as a valuable aid for control in an open-loop scheme if a suitable process model exits. Several examples of such applications have been indicated.

In a case story from a production plant some of the points made above about system design and about programming systems will be documented.

Two trends for the future of control of bioprocesses are seen. The first trend is an extension of the development from the control of single process units to an engineering optimization and further to an economic optimization of the process. Work is in progress to develop plant and business management systems covering multiple plants and observing several months or even years.

A second trend in the application of computers is the use of self-tuning controllers and expert systems to replace or to compensate for the lack of detailed process and plant models. Both of these trends hold promise for future applications in the biotechnical industry.

BIO Technica'85 Hannover

SOME TOPICS ON COMPUTER-AIDED OPERATION OF BIOCHEMICAL REACTION PROCESSES

by

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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 more obious, but also in research laboratories. The computer provides fast and efficient data aquisition, ability to monitor and control experimental conditions, and flexibility in the operation of the fermenter.

In the presentation, some of aspects of computer-aided operation of biochemical reaction processes will be shown. Especially, topics will be focussed on the control of the specific growth rate in the baker's yeast fed-batch culture.

First, an advanced control system which utilizes a simple mathematical model and a PID feedback controller, including automatic parameter tuning, was developed for a baker's yeast fed-batch 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 objective of the control system is to maintain a maximum feed rate of sugar without ethanol production.

If there is no ethanol production, the ethanol concentration does not chenge. Then, the ultimate goal of the control system is to maintain the ethanol concentraiton at a constant value in order to attain maximum volumetric productivity and cell yield.

The cell concentration increases remarkably during the cultivation process. Then, the problem of parameter adjustment of a PID controller with an increase in the cell concentration and a sudden change in feeding conditions, such as pump capacity and input glucose concentration, must be considered when constructing a successful control system for fed-batch cultures. The developed control system was given as a combination of the exponential 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 was tuned automatically based on the given function of the cell concentration in the feeding medium. The advanced control algorithm proposed here was shown to be valid experimentally.

Second, the results obtained here was extended to a practical and useful computer control scheme so that a state variable or a key parameter will as accurately as possible follow a desired profile specified in advance. For example, by the control scheme the specific growth rate can be followed not only the maximum constant value but also a given

arbitrary profile. The control scheme called "Programme-controller/ Feedback-compensator(PF) system", was proposed. This control system consists of a programmed controller which should follow the desired profile unlesss there is noise or disturbance, and a feedback compensator which should compensate the disturbance and the noise. As the feedback compensator, the Model Reference Adaptive Control(MRAC) algorithm was also proposed. 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 PI feedback controller was also adopted as the feedback compensator, and the whole 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 verified the usefulness of the proposed control system using an experimentally identified mathematical model. PF-MRAC was better than PF-PI for controlling the error of the specific growth rate deviated form the desired profile.

Finally, the proposed profile control of the specific growth rate was realized experimentally in a baker's yeast fed-batch culture. For this, the specific growth rate itself must be observed or estimated. For the range of low cell concentration, the cell concentration can be measured by a type of turbidity meters, such as UV-photospectrometer, which was used in our experiment. And the specific growth rate can be estimated using the observed cell concentration. For the range of high cell concentration, there is no practically available sensor for cell concentration without sampling and diluting. Then, another technique, e.g., 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 laboratory scale fermenter. And the results showed that the estimation and control scheme were sufficiently useful for the profile control of the specific growth rate.

BIO Technica'85 Hannover

COMPUTER BASED MANAGEMENT OF BIOTECHNICAL PROCESSES

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Fermentation processes have certain specific features, which also influence the requirements on computer control systems. In some respects these differ from standard automation systems applicible to chemical processes in general.

The following features, which are typical for all types of biotechnical processes, strongly influence the properties required by a computer system.

- Batchwise production
- Comparatively slow process dynamics
- Recipe based operation with different stages such as charging, sterilization, inoculation, fermentation, harvesting etc. which all have their own control programs.
- Important to store historical data for later use.
- Laboratory analyses also to be stored are received with big delays sometimes when on-line operation is terminated.
- Lack of on-line sensors limits control possibilities, puts requirements on estimation algorithms.
- Production of pharmaceutical products sets strict requirements (GMP) on documentation and logging functions.
- Contaminations appear easily, may spoil whole batches.
- Reproducibility is difficult under manual conditions. small deviations in environmental conditions influence result.
- The products and raw materials are often very valuable.
- The differences between laboratory, pilot and production plants are often diffuse.

The whole range of fermentors may exist in the same unit and it is an advantage if all types of fermentors and also down stream units can be connected to the same computer system.

The importance of certain features is, however, dependent on whether the system is to be used in laboratory, pilot or production scale plants.

It is, however, often difficult to clearly distinguish the different plant types as production of certain products may take place in 10 l laboratory fermentors. It is therefore an ideal situation if the same basic computer system can handle all types of fermentation processes and differences only are considered by choice of subsystem consisting of process interface and controllers.

The following table gives a rough classification of certain features in research plants on the one hand and production plants on the other hand.

	Research	Production
Data acquisition	++	++
Entering laboratory analyses	++	++
On-line calculation	++	++
Post calculation	++	-
Mathematical models	++	-
Direct digital control (DDC)	+	++
Set point profiles	++	++
Sequence control	-	++
Alarm handling	+	++
Monitoring displays	+	++
Curve displays	++	++
Recipe based operation	+	++
Alphanumerical reports	+	++
Graphic reports	++	++
Logging of operator's action	+	++
Long term storage	++	++
Graphic comparison of experiments	++	+
Production planning	-	-

++ = Very important
+ = required sometimes
- = not required

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The overall computer-based management of biotechnical processes requires a flexible system which can handle several different process units running experiments with different recipes at the same time.

Figure 1 shows a scheme of the different stages of fermentation processes from the point of view of data file processing. The on-line operation stage includes all the different fermentation stages.

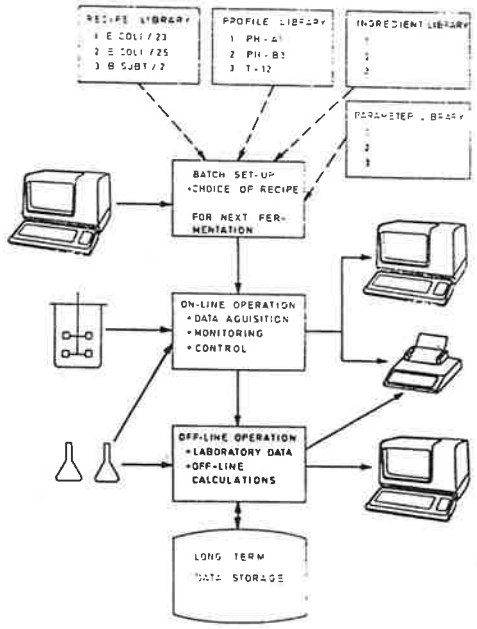

Figure 1: Scheme of file treatment during a fermentation cycle.

By utilizing a computer system with a clear data file structure and sufficient mass storage, extensive documentation in accordance with GMP-requirements, but also suitable for scientific purposes, can easily be obtained.

RECOVERY OF BIOLOGICALLY ACTIVE PROTEINS

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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 and also 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 since the specific interactions with receptors or substrates occur in molecular dimensions. This fact has two consequences:

1. The biosynthetic machinery of living cells is needed for the industrial production of proteins, since a chemical synthesis lacks the necessary precision and economy.

2. The native structure of biologically active proteins must under all circumstances be preserved (or restored) during isolation and purification. This makes the task to separate one desired protein from a complex mixture in general quite difficult and limits applicable environmental conditions and exposure time.

The degree of purification required for a given protein will depend on its ultimate application. While technical catalysts require only certain enrichment and absence of few interfering enzymatic activities, a considerably higher purity is requested for enzymes employed for analytical or genetic engineering purposes. The highest specifications have to be met with proteins for therapeutical use, especially those which are intravenuously applied.

BIO Technica'85 Hannover

The general scheme followed in the downstream processing of proteins is shown in the fig. The methods utilized will depend on the scale of operation. While in the laboratory methods of protein purification are developed to a high degree of sophistication and perfection, there are considerable gaps in biochemical engineering studies of the unit operations involved and problems encountered in the processing of large amounts and/or volumes in industrial scale.

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 the efficiency of the single steps improved. Modern developments lead 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.

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BIO Technica'85 Hannover

ENZYME-CATALYZED PROCESSES IN ORGANIC SOLVENTS

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One of the main obstacles in the utilization of enzymes as catalysts in organic chemistry is the commonly assumed necessity of conducting enzymatic reactions in aqueous solutions. We have recently discovered that many different enzymes (hydrolases and oxidoreductases) can vigorously act as catalysts in a number of nearly anhydrous organic solvents. Such enzymatic reactions obey Michaelis-Menten 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.

Some enzymatic processes can be carried out on a practical scale only in organic solvents, for they are nearly impossible in water due to kinetic or thermodynamic reasons. Preparatively important examples of that, developed in our laboratory, include (i) production of optically active compounds via lipase-catalyzed stereospecific esterifications and transesterifications, (ii) lipase-catalyzed regioselective acylation of glycols, and (iii) polyphenol oxidase-catalyzed regiospecific hydroxylation of phenols. Prospects of these and other biocatalytic transformations in organic media will be discussed.

ENZYME ENGINEERING

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Enzymes and other proteins such as antibodies, can now be altered to order 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 arginine residues has allowed the mapping of the path of the tRNA across the surface of the synthetase. However, as well as such academic applications, this technology may permit the tailoring of enzymes for industrial or medical use. Already it has 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. The rationale design of improved enzymes will be reviewed.

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Ethanol Production by Immobilized Cells

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With respect to ethanol fermentation, the batchwise fermentation process is still dominant today. However, in order to produce fuel alcohol more economically, it is necessary to attain much improved productivity and reduced manufacturing cost in comparison with the conventional process. For these requirements, several processes such as an yeast cell recycling system and a flocculated cell system have been proposed. RAPAD organized under the auspices of Ministry of International Trade & Industry of the Japanese Government decided to develop 2 kinds of continuous fermentation processes using immobilized growing yeast cells according to the recent development of immobilized cell technology. One is the process using the immobilized yeast gel prepared by calcium alginate. Another is the process using the immobilized yeast gel prepared by a photo-crosslinkable resin. The basic research had been already done and the research on the pilot plant scale has been carried out now. For large-scale production, the following should be satisfied: 1) good conversion yield by contamination prevention, 2) prolonged viability of the immobilized carrier, and 3) practical operability during the immobilization and fermentation steps. Those results are described in this paper.

(1) The process equiped with immobilized yeast cells entraped by calcium alginate gel

Saccharomyces cerevisiae was used as the yeast. The continuous preparation of immobilized cell beads was carried out by showering drops of sodium alginate solution containing live yeast cells from the top nozzle into calcium chloride solution in the reactors(Fig.1).

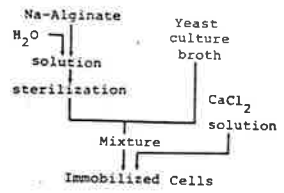

Figure 1 Preparation of immobilized yeast cells.

The content of sodium alginate in the mixture was finally adjusted to 3-4 %. 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 investigations to prevent 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 effective. So, the process became operable without sterilization of the inlet medium.

The process flow as shown in Fig. 2 was employed in pilot operations. This pilot plant was composed of two reactor channels. One channel consisted of two columns(each 1 kL) in series, and the other channel consisted of three columns(0.8 kL for one and 0.6 kL for the other two). The total column volume was 4 kL and the total productivity was 2.4 kL ethanol/day. As a result of the pilot plant operation, 8.5-9.0 %(v/v) ethanol was constantly produced from diluted cane molasses for over 4000 h (ca. six months) as shown in Fig.3. The productivity of ethanol was calculated as ca. 20 g/L total volume/h(33 g/L gel/h). This means that 600 L pure ethanol is produced each day by using a 1-kL column reactor.

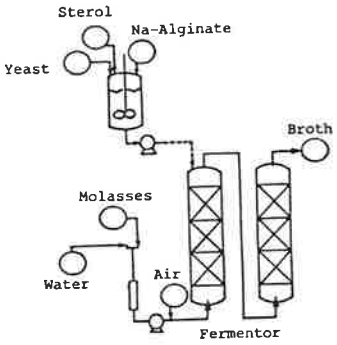

Figure 2 Process flow diagram of the pilot plant operation.

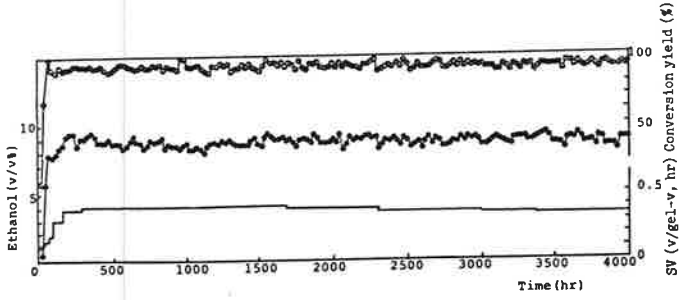

Figure 3 Continuous alcohol fermentation by immobilized growing yeast cells in the pilot plant (run 7): (○) conversion yield, (●) ethanol and (–) space velocity.

(2) The process equiped with immobilized yeast cells entraped by photo-crosslinkable resin

Saccharomyces species was used as the yeast. The photo-crosslinkable resin as shown in Fig. 4 was about 310 Å long and the chain polymer was composed of 65 % polyethyleneglycol moiety and 35 % polypropyleneglycol moiety. The immobilized yeast gel was prepared as follows. The mixture of 40 g photo-crosslinkable resin , 60 g yeast suspension and very small amount of benzoyl peroxide was spread on the plastic sheet and was exposed by light of 300 to 400 nm to produce the 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 the pilot plant is shown in Fig. 5.

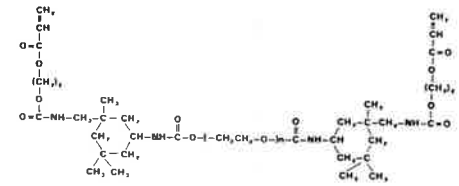

Figure 4 Structure of photo-crosslinkable resin

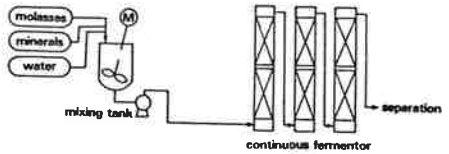

Figure 5 Process flow diagram of continuous ethanol fermentation

Fig. 6 shows the record of the pilot plant operation, covering about 3000 hrs. This figure verifies that the immobilized yeast used in this process was able to maintain stable activity for long period and that contamination was able to be almost completely controled by very low concentration of sodium metabisulfite under non-sterilized fermentation. The cell concentration, final ethanol concentration , yield on sugar, fermentation time and the productivity were 40 g/L, 8.5 g/100 mL, 95 %, 5 hr and 11 kg ethanol/m^3.hr, respectively.

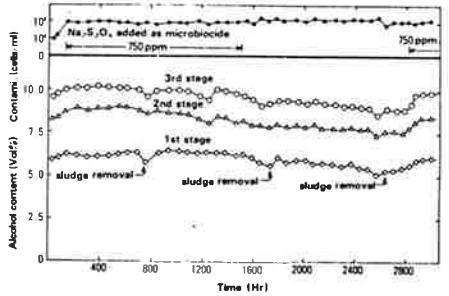

Figure 6 Pilot plant test without sterilization of the inlet medium

ANIMAL CELL CULTURE including MONOCLONAL ANTIBODY PRODUCTION

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Animal cell cultures are often regarded as being difficult to achieve and expensive to produce. The reasons given for such views include:-

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 lage 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,
5. materials produced from animal cells in culture may be carcinogenic because the properties of such cells is such that in order to flourish in culture those genes which produce transforming factors are activated which means that products from such cells may be contaminated with putative tansforming agents.

While most of the contentions outlined above may have seemed to be important in the 1960's and even into the 1970's, the present situation offers evidence to reverse the traditional views. It is now relatively commonplace for animal cell cultures to be run at scales of 8,000 litres in stirred tank reactors. Such systems are in use for the production of alpha-lymphoblastoid interferon from Namalwa cells and for the production of Foot-and-Mouth disease vaccines. There is an active program for the development of air-lift fermenter systems for the production and exploitation of hybridoma cell lines producing monoclonal antibodies at the 1,000 litre scale; a development which could lead to similar types of reactors operating at scales of 10,000 litres or more. Such activities, in the stringent environment of the commercial organisation demonstrate with clarity that the contention that animal cells are delicate fagile entities does not always apply. For each of the systems alluded to, the cells used in the large reactors have been 'hardened' to the environment of the bioreactor by a program of selection and phenotypic adapation and weaning.

Less demanding cell adapation processes have been used to obtain cell cultures which require solid surfaces for their growth. Again, the technology for such systems has, during the last 10 years, been radically transformed. Whereas the traditional system of choice 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 commonly used at the 1,000 litre scale for the production of polio vaccine from Vero cells grown on the surface of microcarriers held in suspension in a stirred tank reactor and there are reports (verbal rather than printed) that in Japan beta-fibroblastic interferon is produced in a similar system at scales of between 5,000 to 20,000 litres.

The large systems referred to above would be uneconomic were they to rely on the use of foetal calf serum (costing about £80 to £100 per litre) to a concentration of up to 10% of the medium. Rather alternative sera can be used which are more than an order of magnitude less expensive. (Adult bovine serum can cost as little as £3-10 per litre while newborn calf serum is more expensive) A further feature of the use of adult sera is that the probability of a viralogical or mycoplasmic contamination due to improperly colected or sterilised 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-Bar 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.

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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 the authors laboratory for the interactive set point control of analogue controllers may be cited as a case in point. It would also seem that present developments in the way in which concentrated cell systems are handled in the animal cell area may prove heuristic to microbial biotechnologists in other areas. While there are many examples the use of gels to provide a matrix for the immobilisation 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 monoclonal antibodies and at present there is a fierce competition between the proponents of microsphere entrappment systems with the alternatives of entrappemt on the "shell side" of a capillary bundle or between the decks of planar membranes held in a stacked or cassetted form.

From the considerations described above, it is reasonable to conclude that much of the mythology of cultivating animal cells has been misfounded. The technological problems have been surmounted and contiue to provide opportunities for exciting 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. One can even report progress on the way in which the products of animal cells are viewed by the regulatory agencies. Understanding hte mechanism of cell transformation and the relations of this phenomenon with oncogenes, retroviruses and growth factors and their receptors will in the future lead to an increase in our ability to use animal cells for the benefit of all.