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Claire E. Zomzely-Neurath

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Presentations in selected topics given at this meeting, held in June 1987, are summarized. Based on the variety and depth of the presentations at this conference as compared to those at the conference in previous years, the author states that biotechnology research in Europe has expanded tremendously and that European scientists are in the forefront of research in biotechnology. Keywords: Bioreactors; vaccines; genetics; Foot-in-mouth disease; Measles; Pertussis toxin; Liposomes;

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CONTENTS

FOURTH EUROPEAN CONGRESS ON BIOTECHNOLOGY: AMSTERDAM, THE NETHERLANDS

1 INTRODUCTION

The Fourth European Congress on Biotechnology took place in Amsterdam, the Netherlands, from June 14 through 19 June 1987. The meetings and extensive exhibitions were held in the RIA Congress Center, which provided excellent facilities for the many varied activities being conducted in close proximity to each other. Fifty countries were represented at the congress with an attendance of over 2000.

The congress format consisted of general lectures, more than 50 minisymposia, and numerous workshops as well as 800 poster presentations. The topics covered at this extensive and informative congress included:

- Biocatalysis
- Animal cell cultures
- Pharmaceuticals
- Environmental biotechnology
- Molecular genetics
- Bioreactors
- Downstream processing
- Fine Chemicals
- Screening and selection for new microorganisms
- Biodegradation of lignocelluloses
- Measurement and control
- Microbial physiology
- Enzymes in biotechnology
- Amino acid fermentations
- Fuel, energy, biomass
- Plant cell cultures
- Protein engineering
- Food (new proteins for men and animals).

There were also some special minisymposia and workshops dealing with the following subjects:

- Biosafety
- Patents
- Developing countries
- Zero gravity biotechnology
- Economics/cost prices
- University/industrial relations
- The biotechnology race (US, Europe, and Japan)
- Biotechnology in the European Economic Community (EEC)
- Raw materials (biotechnology and agricultural surpluses)
- Social impacts and educational aspects of biotechnology.

Since there was such an enormous amount of material presented at this congress it is only possible to cover selected presentations in the various categories listed above. Biocatalysis and downstream processing will not be included in this report as they have been covered in *ECNIB* 88-02:5-11.

2 BIOREACTORS

Aqueous Two-Phase Bioreactors

These systems are obtained by mixing water solutions of two water-soluble polymers. Both phases have a high water content and do not denature biological particles or macromolecules. By changing the composition of the phase system, macromolecules, organelles, and cells can be completely partitioned to one phase. This creates the possibility of using aqueous two-phase systems in biotechnological processes. The bioconversion can take place in one phase while the product can be enriched and extracted with the other.

B. Hahn-Hägerdal and coworkers (Department of Applied Microbiology, Chemical Center, University of Lund, Sweden) studied cellulase production with *Trichoderma reesei* Rutgers C30 in aqueous two-phase systems. The microorganism and the particulate substrate, cellulose, were completely partitioned to the bottom phase. The enzymes were enriched and extracted with the top phase. Semicontinuous cellulase production was carried out by Hahn-Hägerdal and her group for 14 days with intermittent addition of substrate. The average cellulase concentration was arcund 1.5 FPU/ml substrate and the productivity during steady-state conditions was around 20 FPU l/hr.

These investigators also studied yeast fermentation in aqueous two-phase

systems. The fermentation of glucose with *Saccharomyces cerevisiae* and of xylose with *Candida tropicalis*, respectively, were compared in different phase systems and reference media. Polyethylene glycol (PEG) of different molecular weights, fractionated dextran 500, crude dextran, and a hydroxypropyl derivative of starch (Aquaphase PPT) were used as phase-forming polymers. Hahn-Hägerdal said that the volume ratio of the phases have to be chosen so that accumulation of inhibitory concentrations of product is avoided. For technical applications, the price of the phase system has to be considered. It depends both on the phase-forming compounds and the volume ratios.

In a phase system of PEG 20000 and crude dextran the yield of ethanol from glucose in fermentation with *S. cerevisiae* was approximately the same as in a reference medium. Also, in the fermentation of xylose with *C. tropicalis* similar product formation patterns were obtained in the phase system and in the reference medium.

Fluidized-bed Bioreactors

The topic of fluidized-bed fermenters as novel bioreactors for process intensification was discussed by M.J. Dempsey (Department of Biological Sciences, Faculty of Science and Engineering, Manchester Polytechnic, Manchester, UK). Dempsey said that historically, the largest increases in the productivity of industrial fermentations were obtained by the isolation and induction of overproducing mutants. Later in fermentation process development, smaller increases were achieved by the optimization of both growth-media formulations and downstream processing techniques, and by minor modifications to conventional fermenter design. More recently, the use of computers for process control has also led to improvements in productivity. Despite the current battery of techniques for increasing productivity, it is only possible, according to Dempsey, to achieve relatively small improvements using the conventional stirred tank fermenter once the quantum leaps of initial mutations have been obtained. An additional method of achieving a large increase in productivity is by a radical alteration in fermenter design, allowing substantially higher biomass concentrations with some form of cell immobilization. Additionally, the techniques of cell retention also open up the possibility of producing secondary metabolites in continuous culture.

A variety of techniques now exist for the retention of biomass within fermenters, according to Dempsey. These techniques include artificial entrapment of cells inside gel-beads, natural entrapment within biomass support particles, and natural attachment to inert support particles. One major drawback of gel-entrapment methods is that the resultant particles have a limited life due to the eventual death of the cells. Fortunately, this problem is not encountered with either biomass support particles or inert support particles. This is because both dead and live cells are continuously lost from these particles, and any resulting spaces are rapidly recolonized. In this way, the immobilized biomass particles have an unlimited life, because the cells associated with them are continuously replenished.

Furthermore, according to Dempsey, inert support particles have an additional advantage compared to biomass support particles. This is linked to their respective sizes. With biomass support particles the lower size limit is about 6 mm, due to constraints of the manufacturing process; whereas much smaller inert support particles are available because these are naturally occurring materials such as sand or coke. Typically, these natural materials are used in the size range 0.25 to 2.0 mm, which is ideal for use in fluidized-bed fermenters.

According to Dempsey, the operation of an immobilized biomass fermenter as a fluidized bed has significant process advantages, in addition to those gained by using cell retention. These additional advantages stem from highly efficient mixing, which is characteristic of the flow patterns induced by fluidization. The composite particles, of biomass-coated inert supports, are relatively small; and when these are fluidized by

the growth medium, the metabolized nutrients are replenished and the formed products are removed. In this way, the diffusion path for both nutrients and products is limited to the thickness of the attached film.

Although sand has been the material traditionally used for the support particles in fluidized bed fermenters, it is considered that coke, according to Dempsey, is more suitable because, although equally robust, it is also porous. These pores provide a protected environment for the initial attachment of cells and also lower the density of the particles. This reduces the upward velocity required for fluidization. In addition to the resultant lower pumping-energy requirement, there is also a reduction in the shear forces at the surface of the coke. According to Dempsey, the lower fluidizing velocity, the reduced shear forces, and porosity combine to make coke a superior support material for the natural immobilization of microorganisms in fluidized bed fermenters (FBF).

The high biomass concentrations and efficient mixing characteristics of FBF's combine to produce bioreactors of high productivity, according to Dempsey and coworkers. These investigators found a sixfold increase in the volumetric productivity of a FBF employing *Z. mobilis* naturally imobilized on coke when compared to that predicted for a conventional stirred tank fermenter operating with freely suspended biomass. Similar results were obtained by Dempsey and his group with the yeast, *Saccharomyces cerevisiae*, and the actinomycete, *Thermomonospora fusca*.

So far, successful FBF's have been developed at the laboratory scale for unicellular yeast (*S. cerevisiae*) and unicellular bacteria (*Z. mobilis*). However, one drawback with these organisms was the necessity of finding a suitably adhesive strain before high concentrations of biomass could be obtained. This has not been a problem with the filamentous bacteria (actinomycetes). Dempsey and his group have so far tested four cultures in FBF, and all were able to attach to the support particles and produce high biomass concentrations. While these actinomycetes were all species of *Thermonospora*, Dempsey does not think that the the genus is important in conferring the ability to attach and form thick films. Rather, he thinks that the attachment and film-forming ability is a common property of most actinomycetes, being conferred by their filamentous morphology. Therefore, Dempsey thinks that other, more commercially important actinomycetes will be easy to immobilize on coke, thereby leading to the development of highly productive FBF's. For example, the antibiotic-producing species would be likely candidates. Dempsey said that if it is possible to produce such high-value secondary metabolites in FBF's, then it should also be possible to operate the fermenters in the continuous culture mode, thereby further improving the system's productivity.

Dempsey said that at present, a variety of systems are being developed with the aim of evaluating the range of organisms and products for which this exciting technology is appropriate.

Membrane Bioreactors

The continuous hydrolysis of triglycerides in a membrane bioreactor was reported by W. Pronk (Department of Food Science, Food and Engineering Group, Agricultural University, Wageningen, the Netherlands). Enzymes of the lipase class can be used for the hydrolysis of oils and fats in the large-scale production of fatty acid and glycerol. Water and oil are not miscible, so this reaction will take place at the water/oil interface. At the industrial scale, according to Pronk, an emulsion is formed to which the lipase is added. Immobilization of lipase is a method to prevent enzyme loss.

Pronk and his group used a membrane to immobilize the lipase enzyme. The reaction takes place at the interface, so the active surface area is rate limiting. These researchers used cellulose-type membranes with a hollow fiber configuration having a specific area of about 10,000 m^2/m^3. An additional advantage of this process, according to Pronk, is that

both phases are kept separate; emulsification is not necessary, and the products, glycerol, and fatty acid can simply be recovered from two separate streams.

Pronk and his coworkers carried out several batch and continuous experiments which gave information about the kinetics of the reaction. In continuous experiments, the stability was examined under various conditions. The half-life time for the lipase was found to be almost 50 days at a temperature of 30°C. An increase in temperature resulted in a smaller stability, while the initial activity was increased. Pronk and his group also carried out a temperature optimization study. These investigators found that the amount of immobilized lipase determines the activity of the reactor. However, since the surface area is a rate limiting factor, it is to be expected that the activity reaches a maximum at increasing enzyme-load.

Variable Surface Packed-Bed Reactors

This topic was discussed by P.P. Matteau (Division of Biological Sciences, National Research Council of Canada, Ottawa, Canada). According to Matteau, the use of packed-bed reactors for bioconversions using immobilized cells or enzymes has the advantage of simplicity of operation, high mass-transfer rates, and high volumetric reaction rates (for processes which are not inhibited by the substrate concentration). On the other hand, a unique feature of the packed-bed reactor is that there exists a pressure drop or resistance to flow across the reactor.

One approach to sucessfully circumvent this major operational difficulty has been the development of two novel packed-bed geometries; a radial-flow packed-bed and a tapered packed-bed reactor. Both the geometries, according to Matteau, present an increase in bed surface area in the direction of flow resulting in a drop in fluid velocity and a reduction in the pressure drop. Initial theoretical analysis of pressure drops across packed-beds indicated that relative to tubular reactors the pressure drops in tapered beds are lower by one order of magnitude whereas in radial-flow reactors a reduction of greater than two orders of magnitude can be achieved for similar liquid flow rates.

Comparative studies with two reactors on each of the three geometries (tubular, tapered, and radial) were carried out by Matteau and his group using two bioconversion processes: an enzymatic conversion (cellobiose to glucose using calcium alginate immobilized *Trichoderma harzianum* E58; and a gas-producing fermentation (ethanol fermentation using calcium alginate immobilized *Saccharomyces cerevisiae* NRCC 202076). Reactor volumes were fixed to 1 liter and the length-to-diameter ratios were varied for each configuration to evaluate both the kinetics and the operational stability of each reactor type.

For the enzymatic biotransformation, few operational difficulties such as bed compression, channeling, or excessive pressure drops were encountered in any of the reactors. Additionally, the steady-state conversion data were identical for all reactors tested using substrate concentrations of 2 to 30 mM and dilution rates varying from 0.1 1 to 3.5 1/h. The results confirmed that the total enzyme activity in each reactor was the same and that no obvious channeling occurred.

For the fermentation, tests were carried out at glucose concentrations ranging from 1 percent to 12 percent and dilution rates from 0.2 to 2.7 1/h. Extensive bed separation, channeling, and high pressure drops were encountered using the tubular reactors. On the other hand, both variable-surface reactor geometries were found to operate with little difficulty for the duration of the experiment (25 to 30 days). Matteau said that the conversion efficiences in these reactors were also consistently higher than those obtained in tubular reactors.

Biosensors

The topic of of nanomolar sensitivities of biosensors with the use of recycling immobilized systems was discussed by D. Kirstein (Central Institute for Molecular Biology, Academy of Science of

the German Democratic Republic, Berlin-Buch, East Germany). This was a collaborative study carried out with B. Danielsson, F. Scheller, and K. Mosbach (Department of Pure and Applied Biochemistry, Chemical Center, University of Lund, Sweden). The latter three scientists are well known for their research on biosensors. Kirstein said that the introduction of cofactor and substrate recycling systems in enzymatic analysis has extended the sensitivity of metabolite detection in biological fluids. The apparent advantages of a flow-through system consisting of a thermistic detector and one or more enzyme column reactors can be used to build up an automated analytical device with high sample throughput and precision.

Kirstein described the preparation of a multienzyme reactor by coimmobilization of enzymes of a cofactor or substrate recycling system and its combination. An increase of ADP/ATP detection by three orders of magnitude was achieved by introduction of a double recycling system. In this case, an additional enzyme reactor was connected in series with the reactor which was part of the enzyme thermistor (ET) unit. The first reactor contained pyruvate kinase (PK) and hexokinase (HK) coimmobilized on controlled-pore glass (CPG) catalyzing ADP-ATP recycling according to cycle A in Figure 1. Pyruvate, the product of this first cycle, was recycled in the second enzyme reactor (inside the ET) containing the enzymes of cycle B (Figure 1), L-lactate dehydrogenase (LDH), lactate oxidase (LOD), and catalase. This latter system had been described previously by the group at the University of Lund, for lactate determination.

Ammonia contamination of the enzymes was removed by gel filtration on Sephadex and then the immobilization was performed on aminopropylated CPG via glutaraldehyde at pH 7. To determine the efficiency of cycle A (Figure 1) the PK/HK-reactor was introduced into the ET-unit, affording the possibility of measuring the reaction enthalpies of kinases. Calibration curves for ADP and ATP were determined without recycling--i.e., in the absence of the

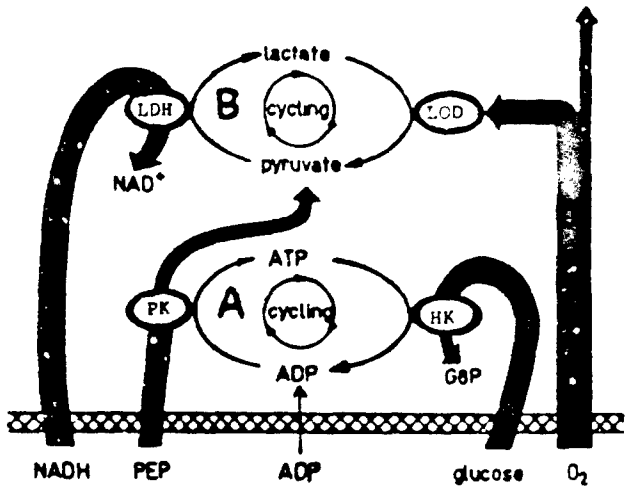


Figure 1. Scheme of the double cycling system, A: ADP/ATP recycling B: pyruvate/L-lactate recycling; pyruvate is a product of cycle A.

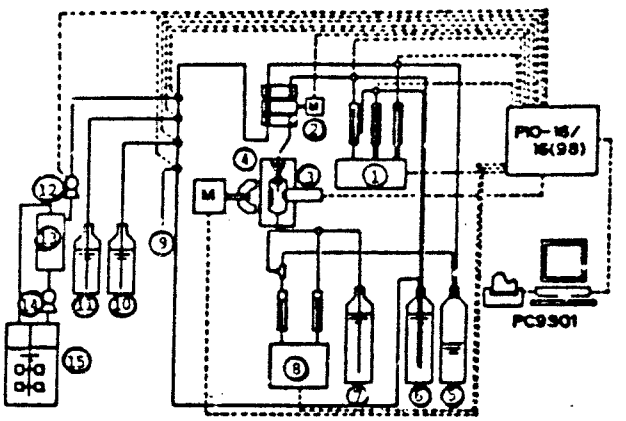
substrate that is necessary for cofactor recycling (glucose for ADP and phosphoenolpyruvate [PEP] for ATP). HK gave a negative reaction enthalpy in the buffer used (Tris, 30 mM Mg^{++}, 10 mM K^{+}) whereas PK gave a positive one (temperature decrease). It was found that for both enzymatic reactions, the thermistor signal depended linearly on the cofactor concentrations. ADP or ATP measurement under recycling conditions in the presence of PEP as well as glucose led to an increase of the sensitivity. Despite the positive enthalpy of the PK reaction, the overall enthalpy of cycle A is negative and reaches the same temperature readings at 30-fold lower cofactor concentrations as in the HK-reaction without recycling. Kirstein said that there was no difference whether the reaction was started by ADP or ATP. In the double recycling system the pyruvate leaving a PK/HK precolumn was measured by recycling in the LDH/LOD/catalase-reactor as part of the ET-unit. A further amplification by the second cycle was obtained, yielding an overall recycling factor of about 1700. Thus, a decrease of the lowest limit of detection was achieved for ATP (ADP) from 60 uM in a pyruvate kinase-hexokinase-reactor without amplification, to 2 µM

with cofactor recycling and to 10 nanomolar in the double recycling system with cofactor and pyruvate cycling.

On-Line Control of Glucose Concentration in Fed-Batch Cultures

This subject was discussed by T. Kobayashi (Department of Chemical Engineering, Faculty of Engineering, Nagoya University, Nagoya, Japan). Kobayashi said that carbon source is one of the most important medium components in fermentation. However, there have been few appropriate sensors available for the detection of carbon sources, which has limited the control strategy and the optimal use of carbon sources. The only successful case, according to Kobayashi, has been for a volatile carbon source such as methanol. He and his group have developed a methanol sensor and showed that the methanol concentration could be controlled at a constant level for the cultivation of a methanol-utilizing bacterium. However, few sensors have been reported for direct measurement of a non-volatile carbon source such as glucose, which is often used for the cultivation of microorganisms. Thus, glucose has been fed in a medium by empirical methods such as assuming constant biomass yield or by using a drastic increase in dissolved oxygen concentration as an indicator of the sugar starvation. In this report, the fundamental characteristics of a glucose sensor newly developed by Kobayashi and his group were examined and applicability of the sensor to on-line control was investigated.

The microorganisms used in this study were *Saccharomyces cerevisiae* and *E. coli* C600 harboring a recombinant plasmid from the tryptophan promoter gene and the β-galactosidase gene. The schematic diagram of the automatic glucose analyzer is shown in Figure 2. Culture broth was filtered by a membrane filter (0.2 μm) and the filtrate was withdrawn by a sample syringe drive unit, a part of which (15 μl) was sampled by a slide valve and injected by the unit. A glucose sensor which consisted of an immobilized glucose oxidase membrane and an oxygen sensor was installed in the measuring



1. Sample syringe drive
2. Slide valve
3. Glucose sensor
4. Measuring cell
5. Bottle for waste
6. Bottle for water
7. Bottle for the phosphate buffer
8. Buffer feed/drain drive unit
9. Air inlet unit
10. Standard 1
11. Standard 2
12. Pump for removing the filtrate
13. Filtration unit
14. Pump for recycling the broth to jar-fermentor
15. Jar-fermentor

Figure 2. Schematic diagram of the automatic glucose analyzer.

cell. Output voltage of the sensor was differentiated twice in order to obtain a quick response, and its value was compared with those of the standard glucose solutions. The whole procedure of the sampling and the glucose analysis was fully automated. Off-line glucose concentration was measured by Glucostat reagent (Worthington Biochemicals Co, US). Ethanol concentration was measured with a gas chromatograph.

Initially, the relationship between glucose concentration and output voltage of the analyzer was examined for various glucose concentrations. The output voltage was linear up to 10 g/l. Fed-batch culture of *S. cerevisiae* with on-off control of glucose concentration using the automatic glucose analyzer was carried out. The glucose concentration was controlled at 10 g/l. The organism grew exponentially to optical density at 570 nm (OD) = 43 and the specific growth rate

was 0.41 1/h. Glucose concentration was kept at almost 10 g/l through the cultivation, and the fluctuation was within 10 percent. Kobayashi said that the concentrations measured on-line coincided well with those obtained from off-line measurement. Ethanol was produced exponentially by the Crabtree effect and the final ethanol concentration was 13 g/l. When the glucose concentration was set at 0.3 g/l it was again found that the glucose concentration measured by the on-line system was essentially the same as that obtained by the off-line measurement. In experiments using *E. coli* C600, it was found that the specific activity of ß-galactosidase was increased to 25 U/mg of protein in the system used by Kobayashi and his group.

Flow Injection Analysis

A flow injection analysis system for on-line fermentation monitoring and control was discussed by M. Garn (Central Analytical Department, CIBA-GEIGY Ltd., Basel, Switzerland). Garn said that during fermentation the concentration of nutrients, especially the limiting one, have traditionally been measured by off-line assays or estimated from on-line measurements of secondary compounds--oxygen, for example. According to Garn, on-line discrete and specific analysis of these compounds gives more reliable results for fermentation control than the other techniques as almost real-time results can be given. In addition, changes in instrument and surrounding conditions--for example, temperature jumps, baseline drift, and pollution--are automatically corrected.

A fermentation monitoring system for on-line determination of nutrients based on the Flow Injection Analysis technique was presented by Garn. The system includes continuous on-line filtration, dynamic gradient dilution, autocalibration during monitoring, enzymatic conversion, and colorimetric detection. The controlling and data acquisition are made by the same computer. According to Garn, this makes possible feedback information to the controller of the measured concentrations, which again can trigger changes in analysis frequency and sample dilution. The instrument can be unattended for monitoring of nutrients--glucose, for example--over two concentration decades during whole-batch fermentations. The same concept can be used to monitor product formation, according to Garn.

Flow Injection Enzyme Immunoassay

This subject was addressed by B. Mattiasson (Department of Biotechnology, Chemical Center, University of Lund, Sweden). According to Mattiasson, immunochemical binding assays have many properties which make them attractive for their application in process control. One severe limitation has been the time needed for an assay. The binding step is most critical in enzyme-linked immunosorbent assay (ELISA). However, the various manually operated steps cause variations in the results. The methods used to minimize these has been to allow the binding to reach equilibrium. On the other hand, if the errors due to experimental handling could be reduced, there would be no need to use equilibrium conditions, according to Mattiasson. By applying the principle of continuous flow assay, Mattiasson and his group have shown that nonequilibrium assays were fully acceptable and had a time requirement of 12 to 13 minutes for an assay cycle.

Modern flow-injection analysis (FIA) is based on the same concept, but here the dead volumes are reduced, different experimental steps are easily automated, etc. Thus, according to Mattiasson, a flow-injection ELISA could be an improvement over the existing immunoassays. Therefore, these investigators studied the analysis of transferrin in human plasma using an immobilized preparation of rabbit antihuman transferrin antibodies and a peroxidase-labeled transferrin. A flow injection system as shown in Figure 3 was used. The antibody column had a volume of 200 µl and the assay was followed using a Shimadzu spectrophotometer equipped with a flow cell of 30 µl volume. The flow system was set up with connections to the binding buffer which was 0.1-M sodium phosphate (pH 7.0, 0.15-M NaCl), as well as the dissociating

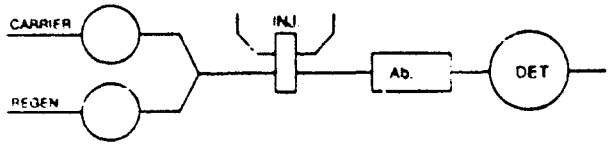


Figure 3. Schematic representation of the FIA system.

buffer via a 3-way valve. The sample and the substrate were injected into the buffer stream through a 200-µl injection valve. The antihuman transferrin antibodies were isolated from rabbit antiserum by first precipitating with ammonium sulfate at 60-percent saturation. Transferrin (Sigma, St. Louis, Missouri) was coupled to Sepharose CL-4B (Pharmacia, Uppsala, Sweden) using the cyanogen bromide coupling method. The affinity gel containing 3.9 mg transferrin/g wet gel was packed in a column and was equilibrated in 20 mM Tris-HCl buffer, pH 7.5. The antibody preparation suspended in the same buffer was applied to the column. The unbound material was washed off prior to elution of the antibodies with 0.2 M glycine-HCl, pH 2.2. The pH of the eluted antibodies was changed to 7.0 before being coupled to Sepharose CL-4B. The immunosorbent obtained contained 1.8 mg antibodies/g gel (wet weight). The gel was diluted with native Sepharose prior to packing in the column in the analytical system. Transferrin was conjugated to horseradish peroxidase (HRPO) via activation of the carbohydrate chains by means of sodium periodate.

According to Mattiasson, the need for fast and reliable immunoassays led him and his group to investigate the possibility of applying the ELISA-technique in continuous flow systems and in combination with FIA as reported here, thereby creating conditions for reliable non-equilibrium immunoassays.

In their procedure, a competitive assay is set up where enzyme-labeled antigen has to compete with native antigen for a limited number of binding sites on the antibody column. Mattiasson stressed that it is important to use an appropriate amount of enzyme-labeled antigen. To optimize this, a series of dilutions of the conjugate were tested. It was found that, normally, an amount equivalent to a dilution of 1:1000 was appropriate. Mattiasson also said that another important point when setting up a quick immunoassay based on the reuse of antibodies is the time needed for splitting off bound antigen from the antibody column before reconditioning the column to make it ready for another cycle. For dissociating medium, the buffer was the same as for elution during the purification of the antibodies--i.e., 0.2 M glycine-HCl, pH 2.2. This means that antibodies with a higher affinity that would not dissociate from the antigen at 0.2 M glycine-HCl, pH 2.2, are already removed. Washing pulses of varying duration were tested, and the result was evaluated in terms of remaining enzyme-labeled antigen on the antibody column. The enzyme was quantified based on its activity. After washing for 2.5 minutes, only 1 percent of antigen molecules remained on the column. This value was regarded as acceptable in this study by Mattiasson and his group. They found that the shorter the regeneration time, the faster is the analysis and the shorter the exposure of the antibody preparation to denaturing conditions. It was found that transferin was quantified in the concentration range of 25 to 750 µg/ml (2.9×10^{-7} to 8.8×10^{-6} mol/l). It was possible to quantify transferrin down to 10^{-8} mol/l.

Mattiasson said that a crucial point in such an assay is the stability of the antibody preparation. When testing for the antigen binding capacity by passing a fixed amount of enzyme-labeled antigen, a clear decrease was observed. However, when assaying a fixed concentration of active antigen in the binding assay, a competitive displacement of enzyme-labeled antigen was observed at a constant percentage--quite an unexpected result, according to Mattiasson. Thus, once a calibration curve is set up, this is valid for the "lifetime" of the antibody preparation. The only calibration needed afterwards is that of the 100-percent value.

Mattiasson said that the flow-injection ELISA described above can be characterized by quick and reliable results from one assay within 2 minutes, and one assay cycle takes approximately 7 minutes; reproducibility, since very few manual steps are involved; easy to automate; and needs only recalibration of the 100 percent value. The calibration curve stays valid during the whole lifetime of the antibody column.

A workshop in the section on Bioreactors dealt with the methodological aspects in bioprocess modeling. The areas covered included (1) bioreactor-performance/intergrating-strategy; (2) kinetic modeling, and (3) scale-up/scale-down approach.

Bioreactor Performance

Optimization of reactor performance for coenzyme-dependent enzyme reactions using computer simulation and experiments was discussed by M.W. Howaldt (Fraunhofer Institute for Biotechnological Processes, Stuttgart, West Germany). Howaldt said that the performance of an enzyme-catalyzed reaction may depend strongly on the kinetics and on the type of reactor employed. Therefore, the suitability of the two continuous reactors--(CSTR) stirred tank reactor and plug flow reactor (PFR)--was studied in theory and experiment by Howaldt and his group. For the glucose dehydrogenase (GDH) and mannitol dehydrogenase (MDH) system the cofactor is continuously regenerated during the reaction and has to be supplied only in catalytic amounts. Howaldt said that the kinetics of each enzyme is adequately described by an extended Michaelis-Menten type equation.

Both the CSTR and the PFR are operated in the recycle mode. Using soluble enzymes, the reactors are followed by an ultrafiltration unit where the low-molecular-weight products are separated from the catalysts.

For the simulation, the system is divided into three compartments: the reactor, the membrane unit and the recycle. The resulting system of eight coupled first-order differential equations is solved using a finite difference scheme according to Gear. Within the filtration unit the enzyme is concentrated--i.e., there are different enzyme concentrations in the three compartments. The lower the recycle ratio, R, the more pronounced the effect is. The CSTR may be operated at high R without changing the performance characteristics so that this effect is negligible, according to Howaldt. In the PFR, however, the recycle ratio and the relative volumes of the three compartments strongly influence the efficiency. For each physical configuration there exists an optimum R.

Howaldt said that the results from experiments with a CSTR and a PFR were in good agreement with the predictions from the model. The importance of the volume ratios in the three compartments was experimentally verified. The influence of the Peclet number as defined in the one-dimensional dispersion model on the agreement between theory and experiment was studied.

Howaldt said that computer simulations are a valuable tool for determining the optimum operating point of a given reactor. For known kinetics and physical dimensions of a proposed membrane reactor configuration the simulation allows the decision whether a CSFR or a PFR should be chosen.

Reactor Development for Immobilized Enzyme Reactor Systems. This development was addressed by H.J. Vos (Department of Biochemical Engineering, Delft University of Technology, Delft, the Netherlands). Vos said that the development of several immobilization techniques has led to a strong emphasis in enzyme process engineering. Due to immobilization, the washout of the biocatalyst can be avoided and some chemical and mechanical stabilization might occur. Despite the research input, however, industrial applications of immobilized enzymes are rather limited, as shown in Table 1. These processes are mainly carried out in packed-bed reactors.

According to Vos, one of the great promises of enzyme technology is due to the substrate specificity of enzymes: the synthesis of complex organic compounds. Because several consecutive reaction

Table 1

Industrial Uses of Immobilized Enzymes

Enzyme	Product
Aminoacylase	L-Amino acids
Aspartase	Aspartate
Fumarase	Fumarate
Glucose isomerase	High-fructose corn syrup
Lactase	Lactose-free whey or milk
Penicillin acylase	6-Amino penicillanic acid

steps are often needed, these processes can be carried out efficiently using immobilized multienzyme systems which utilize the relatively high product concentrations in the immobilization matrix. Problems such as the difference in stability of the often expensive enzyme preparations and the regeneration of cofactors still prevent large-scale applications in this field, according to Vos. These problems have to be solved for the development of enzyme-catalyzed biosynthetic processes. However, the design of a reactor which considers specific characteristics of the complex biocatalyst such as a relatively short half-life time for deactivation and inhibition phenomena is also important.

Vos said that it is to be expected that a counter-current multistage fluidized-bed reactor with a continuous supply and removal of the biocatalyst might, for some reactions, be superior to the packed-bed reactor. In such a reactor, fresh biocatalyst is fed to the upper compartment. The holes in the plate are larger than the biocatalyst particles. This allows downward transport of the particles by periodically pulsating (inverting) the liquid flow. By frequent pulsation, and thus transport of small quantities, a constant biocatalysts activity on each plate results. Nearly inactive biocatalyst is removed from the bottom. The transport of biocatalyst is shown schematically in Figure 4.

Compared to the packed-bed reactor, the multistage fluidized-bed reactor can offer the following advantages, according to Vos:

- Economical use of catalyst up to low rest activity in one reactor because of substrate and biocatalyst, countercurrent flow
- Reduced pressure drop, so smaller particles can be used and thus higher enzyme loadings can be achieved with a large effectiveness factor
- Constant catalytic activity in each reactor segment resulting in a constant product quality
- Continuous processing
- Less problems with channeling
- Less sensitive with respect to contamination.

Vos and his group set up a reactor model assuming the ideal mixing of the biocatalyst at each compartment and plug flow with dispersion for the substrate solution. Internal/external diffusion limitation and possible inhibition effects of substrate and product for the biocatalyst were accounted for. Using this reactor model, biocatalyst activity and concentrations of substrate and product can be evaluated for the compartments, according to Vos.

For verification of the above model, a laboratory-scale 10-compartment reactor with a 0.035-m internal diameter and a height of 2 m was built. Samples can be taken of the substrate solution and the biocatalyst from every compartment. Several model reaction systems are being studied by Vos and his group. Initial experiments using glucose isomerase showed

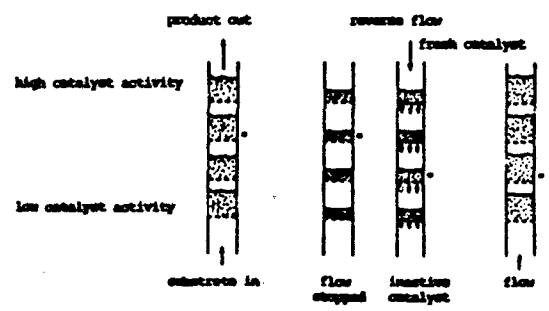


Figure 4. Continuous supply and removal of biocatalyst.

good agreement for the observed and calculated concentrations, according to Vos.

Another model reaction is the hydrolysis of urea with the urease of *Bacillus pasteurii*. A new type of high-density catalyst is under development for this enzyme by Vos and his group in collaboration with the Bio-organic Chemistry Section of DSM Research, the Netherlands. Particle radius and enzyme loading are important parameters for the design of this biocatalyst particle. Vos said that together with a temperature optimization study, this should lead to optimal processing conditions in the multistage fluidized-bed reactor which will be verified experimentally by Vos and his group.

Kinetic Modeling

An improved enzymatic analysis of low-level glucose concentrations in batch and continuous fermentations was reported by C. Brown (Biotechnology Unit, Department of Chemical and Process Engineering, University of Strathclyde, Glasgow, UK). Brown said that the analysis of the growth of *Saccharomyces cerevisiae* in both batch and continuous culture is complicated by the phenomenon variously referred to as glucose repression, catabolite repression, or the Crabtree effect. The traditional view was that catabolite repression was caused by high levels of glucose or other readily metabolizable sugars. However, it has now been shown, using continuous cultures, that it is a more complicated situation. Catabolite repression can occur at low glucose concentrations when there is a high flux of substrate through the cell. In batch culture the initial growth phase yields ethanol, and it is only after most of the glucose has been metabolized that aerobic respiration is initiated. Since during this transition the concentration of glucose is very low, it has not been possible to define precisely the conditions at which it occurs.

In carbon-limited continuous cultures aerobic respiration occurs at low dilution rates. As the dilution rate is increased to a strain-specific critical value, transition to fermentative metabolism occurs and ethanol is formed with a simultaneous decrease in yeast concentration. According to Brown, analysis of this transition is also limited by the relatively high detection limits of available glucose assays. The need for a good and reliable glucose assay technique is further enhanced by the requirement for accurate data to be used in mathematical models to predict the behavior of recombinant DNA fermentations on a large scale.

Brown and his group carried out a detailed analysis of the host strain, *S. cerevisiae* DBY746 in order to facilitate the modeling of a recombinant yeast fermentation, particularly with regard to the kinetic parameters which may be altered due to the presence of recombinant plasmids in the yeast and their encoded functions. *S. cerevisiae* DBY746 is a highly mutated strain previously used by geneticists. In the continuous culture experiments, it was found that residual glucose concentrations could not be measured at the lower end of the dilution rates required since the concentrations were below the analytical detection limits of the commonly used Barham-Trinder assay method.

Brown said that as proper mathematical studies of any fermentation require accurate data, he and his group made a search for other methods to measure residual glucose concentrations of fermentation broths at the low glucose level experienced and which did not necessitate the use of expensive equipment or hazardous materials.

All colorimetric methods used by Brown and his group in their work on glucose determinations have as their bases the following reactions: glucose + oxygen + glucose oxidase + water = gluconic acid + hydrogen peroxide and hydrogen peroxide + oxygen acceptor + peroxidase = a colored product. Brown and his group tested four chromogenic systems: (A) suphonated 2,4, dichlorophenol and 4-amino phenazone; (B) 3-methyl-2-benzothiazolinone (MBTH) and formaldehyde azine of MBTH; (C) 3,3',5,5'-tetramethylbenzidine; and (D), 3-dimethylamino benzoic acid (DMAB) and MBTH.

Table 2

Sensitivity and Detection Limits
of Selected Glucose Assays

Chromogens	Wavelength	Concentration at E=0.03	Sensitivity
A	515	15 mg/l	0.0033 1/(mg/l)
B	670	19 "	0.0021 "
C	450	1.3 "	0.030 "
D	590	0.8 "	0.042 "

Each system was tested by its capability of measuring low glucose concentrations in glucose-water mixtures and its sensitivity to changes in glucose concentrations. The capability of measuring low glucose concentrations was tested, and the concentration giving an absorbance of 0.030 was established. According to Brown, this is often recognized as a practical minimum acceptable value for many spectrophotometers. The sensitivity of the measuring technique was measured as the slope of the standard curve in units of absorbance per mg/l of glucose. After identifying one or more appropriate chromogenic systems, these systems were tested on fermentation broths to evaluate the interference from other chemicals present.

The results of the evaluation of chromogenic systems for the analysis of glucose in aqueous solutions are given in Table 2. The chromogenic systems C and D gave good sensitivity to changes in glucose concentrations and had considerably improved detection limits over the traditional method A. As system B showed no improvement over system A it was dropped from further evaluation. The chromogenic systems A and D were tested in a traditional yeast mineral salt medium and in a fermentation broth without any changes in detection limits or sensitivity. System C, however, was found to be greatly influenced by the mineral salt medium. No color was developed when attempting to measure glucose in such a medium, and when adding small amounts of the mineral salt medium to a system where color already had been developed, this quickly lost its blue color and became turbid. From these observations, it was decided only to test system D fu[illegible]r. The glucose assay technique based o[illegible]hromogenic D was tested on fermentation [illegible]oth both in batch and continuous cultur[illegible]. The fermentation system was a 2-liter stirred tank fermenter operating at a constant temperature (30°C), pH 4.8, and aeration rate (1 liter/min). The batch fermentation medium was 5 g/l glucose, 10 g/l yeast extract and 20 g/l peptone, whereas in continuous culture the mineral salt medium of Oura was used. Twelve ml samples were withdrawn for analyses of glucose, yeast dry weight, and ethanol. The results of the batch fermentation indicated that little if any ethanol was consumed before the glucose concentration became very low. No reduction in ethanol concentration was observed while the glucose concentration was above 1 mg/l. After that the ethanol concentration was found to decrease and at 20 hours was found to be 8 mg/l. According to Brown, this result indicates that the switch from fermentative to aerobic metabolism does occur at very low glucose concentrations. Reproducibility was also tested and found to be very good.

The continuous culture data showed that aerobic respiration occurred below a dilution rate of approximately 0.1 hr. In this range the ethanol yield was very low

(less than 2 percent of the theoretical maximum) and the biomass yield was between 40 and 47 percent of substrate consumed. A sharp change occurred in the fermentation between the dilution rates of 0.092 and 0.125 hr, although there appeared to be no significant increase in the glucose concentration. Between these two dilution rates, the ethanol concentration increased by a factor of 8 and continued to increase as the dilution rate reached a maximum level. Thus, Brown and his group successfully adapted their glucose analysis method for fermentation systems and the procedure was capable of accurately measuring glucose down to 1 mg/l.

Scale-up/Scale-down Approach

Scale-down investigations of the fed-batch baker's yeast production were reported by A.P.J. Sweere (Department of Biochemical Engineering, Delft University of Technology, Delft, the Netherlands). Sweere said that in the performance of large-scale bioreactors, imperfect mixing can play an important role. This may result in a reduction of the biomass growth rate, a change in metabolite production rate, or even loss of viability. Thus, Sweere and his group investigated the influence of mixing effects on large-scale fermentation processes by means of a theoretical process analysis and small-scale experiments. The fed-batch baker's yeast production was chosen as a model system for their studies.

Sweere and coworkers used a reactor system consisting of two interconnected fermenters to impose fluctuating conditions upon the yeast culture. The fermentation broth circulates through this system while the substrate is added in only one of the fermenters. This will cause different substrate concentrations in the fermenters, according to Sweere. The influence of various parameters was investigated. First, Sweere discussed the influence of circulation rate. The two-fermenter system (TFS) consisted of a 5-liter fermenter and a 2-liter fermenter with working volumes of 2.2 and 1.3 liters, respectively. The fermentation broth was circulated through the fermenters by means of a peristaltic pump (0 to 0.35 m^3/min). Glucose was added exponentially to the smaller fermenter according to an equation derived from the biomass, substrate, and volume balance. Samples were taken to analyze the broth for biomass and metabolites. Both fermenters were sparged with air; the gas from the fermenters was analyzed separately for oxygen and carbon dioxide. The specific oxygen consumption rate, which is a measure of the oxidation capacity of the cells could be calculated from the oxygen consumption. Sweere and his group found that on comparing the results in one fermenter with calculated concentrations, the results were optimal for the feeding scheme. From the biomass and ethanol concentrations measured in the TFS, Sweere concluded that imposed fluctuating conditions have a great influence on the fermentation. Even fast fluctuations with a circulation time of 0.6 minutes (viz a mean residence time of 13 seconds in the small fermenter and of 23 seconds in the large fermenter) cause a decrease in biomass yield and an increase in ethanol formation. The concentrations of acetic acid and glycerol were also determined because both metabolites are often produced under transient conditions. In the fermentations in one fermenter, only small amounts of acetic acid and glycerol were produced after 7 hours. In the TFS, formation of these metabolites occurred nearly from the start of the fermentation. However, no relation could be found between the circulation time and the amount of metabolites formed. No difference was found in the biomass and the metabolite concentrations between both fermenters. It was also impossible to detect differences in glucose concentrations, according to Sweere. However, due to the fact that the gas from the fermenters was analyzed separately, differences in metabolism of the cultures in both fermenters could be shown. Thus, it was found that the growth in the fermenter to which glucose had been added was characterized by oxido-reductive metabolism resulting in the formation of ethanol. In the other fermenter, the measured respiratory quotient (RQ) was about 1. In

this fermenter the RQ of the experiment with the highest circulation time showed the smallest difference with the experiment in one fermenter. Sweere said that from all the data obtained in these studies, it appears that relatively fast fluctuations can have a great influence on the production of baker's yeast and the formation of ethanol. However, more experiments are needed to predict the influence of mixing effects on the fermentation at large scale.

Fermenter scale-up using an oxygen-sensitive microbial test culture was reported by U. Saner (Chemical Engineering Department Swiss Federal Institute of Technology [ETH)], Zurich, Switzerland). According to Saner, *Bacillus subtilis* K (AJ1992) provides a sensitive and reproducible method of measuring the influence of reactor parameters on oxygen supply. With the ratio of the two metabolites, acetoin and butanediol, it is possible to characterize the influence of mixing and gasing rates. Due to the reproducibility and sensitivity of the culture, it was possible to distinguish culture responses to small changes in revolutions per minute (rpm) on the 5-liter scale. In three geometrically similar fermenters (Chemap AG) 45-, 450-, and 4500-liter scale-up experiments were carried out keeping the superficial gas velocity constant. During fermentation, the metabolites were analyzed by gas chromatography, and therefore, each stirrer speed could be characterized by metabolite ratio. In addition, information on power uptake and gas analysis were used. Saner said that the results with this biological system indicated that translation to other fermentations and scale-up were feasible.

Mass Spectrometry

A procedure for mass spectrometric measurement in the wheat stalk was reported by G. Langer (Institute of Nuclear Research of the Hungarian Academy of Sciences (ATOMKI), Debrecen, Hungary). Somaclonal plants are said to be more variable and homozygote doubled haploids more uniform phenotypically or in some physiological traits, according to Langer. To confirm this behavior, internal gas composition in the stalks of individual wheat plants regenerated from influorescence and other calluses as well as in control wheat was studied by Langer and his group. These measurements were carried out with a unique analyzer system, consisting of a quadrupole mass spectrometer, fine sampling capillary with silicon rubber membrane, and a growth chamber. The sampling capillary, which is about 1 mm in diameter, is put directly and hermetically in the stem of the plant. The gases in tissues, liquids, and hollows of the plant diffuse across the membrane of the capillary and get directly into the mass spectrometer. According to Langer, *in vivo* continuous multicomponent gas analysis is achieved in this way. The stalk atmosphere contained more water vapor, 20 to 40 times more carbon dioxide, and somewhat less nitrogen and oxygen, than in the lab atmosphere. Its composition was dependent on light/darkness changes and leaf temperature and showed significant inter- and intravarietal variation. The stalk-carbon dioxide probably represents a metabolic fraction, according to Langer. Total overground dry-matter production of the plants was also determined. Wheat plants regenerated from somatic calluses showed a shift towards a higher overground dry mass, but not towards a changed range of CO_2/O_2, while the double haploids exhibited a narrower range of CO_2/O_2 ratio without a population change of overground dry mass.

On-line analysis of fermentations using pyrolysis mass spectrometry was discussed by E.P. Sandmeier (Chemical Engineering Department, Swiss Federal Institute of Technology [ETH], Zurich, Switzerland). The studies were carried out in collaboration with E. Heinzle (Laboratory for Biotechnology and Biochemistry, University of Graz, Austria). According to Sandmeier, mass spectrometry is already a widely used method for continuous on-line analysis of gases and dissolved volatile compounds using a capillary or membrane-covered probe as inlet to the mass spectrometer. Pyrolysis mass spectrometry has already been

used by Sandmeier and Heinzle for off-line characterization of biological materials.

Sandmeier and his group are now developing a continuously operated pyrolysis system which allows analyzing the whole fermentation broth with respect to dissolved or suspended nonvolatile products. The pyrolysis reactor consists of a glass tube equipped with a frit and heated by an electrical furnace up to pyrolysis temperature (400 to 600°C). A carrier gas sweeps the pyrolysis products through a heated capillary into the ionization chamber of the mass spectrometer. The spectrum of all fragments together allows one to draw conclusions about the composition of the initial sample.

Preliminary experiments, carried out with aqueous solutions of sugars, alcohols, and other biochemical reaction related compounds, have shown different spectra for each component and a linear relationship between intensity and concentration. Sandmeier said that further experiments are needed to prove the effectiveness of this procedure for other components such as biomass, proteins, etc. Sandmeier said that a database system will be developed on which calculatory determinations of concentrations in mixtures can be based.

The determination of valine, leucine, methionine, isoleucine, and phenylalanine in fermentation broth by quadruple mass spectometry was reported by G. Santha (BIOGAL Pharmaceutical Works, Debrecen, Hungary). A rapid and specific method was developed by Santha and his group for the simultaneous measurement of five amino acids in fermentation broth by a Q 300 C type Quadrupole Mass Spectrometer. The basis of the method is the reaction of ninhydrin with free amino acids, which is widely used in chromatography for enhancing the sensitivity of detection. The method of Santha and his group measures amino acids as aldehyde derivatives formed in the ninhydrin reaction. In phosphoric acid solution at pH 1.0 and 95°C, valine, leucine, isoleucine, methionine, and phenylalanine yield volatile aldehydes such as 2-methyl-propanal, 3-methyl-butanal, 2-methyl-butanal, 3-mercaptomethyl-propanal, and phenylacetaldehyde, respectively. These aldehydes can be detected by Q 300 C because they are able to penetrate the silicon membrane covering the QMS probe. Acetaldehyde evolved from alanine is also volatile but in the spectrum of an amino acid mixture, it cannot be distinguished from the fragments of the above-mentioned aldehydes, according to Santha. Other aldehydes are not volatile so they do not disturb the determination. A diagram of the determination of amino acids by mass spectrometer is shown in Figure 5.

Santha said that the method he and his coworkers developed can be used for analyzing fermentation broth. The main advantage of the determination is that it does not need any pretreatment of the samples except for filtration. It is simple, rapid, and easy to reproduce, according to Santha. Since the most intensive fragments cannot be used, the detection limit is 10 parts per million (ppm) in fermentation broth or amino acid mixtures. Santha said that it seems possible to reduce the reponse time of the QMS by shortening the distance between the sampling place and the ion source.

3 PHARMACEUTICALS

A general lecture on recombinant strategies in the development of targeted pharmaceuticals was presented by M. Soria (Biotechnological Research, Faramlita Carlo Erba, Milan, Italy). Soria said that one of the most exciting opportunities offered by biotechnology is the potential for combining molecules with diverse functions to obtain desirable biological properties. One example of such manipulations is chemical conjugation of plant and bacterial toxins to monoclonal antibodies to obtain immunotoxins. According to Soria, recombinant DNA (rDNA) techniques can and will be employed to manipulate the genes coding for biologically interesting molecules, with the aim of modifying and combining selected parts of such molecules while discarding other unwanted parts: besides protein and enzyme engineering, domain engineering will

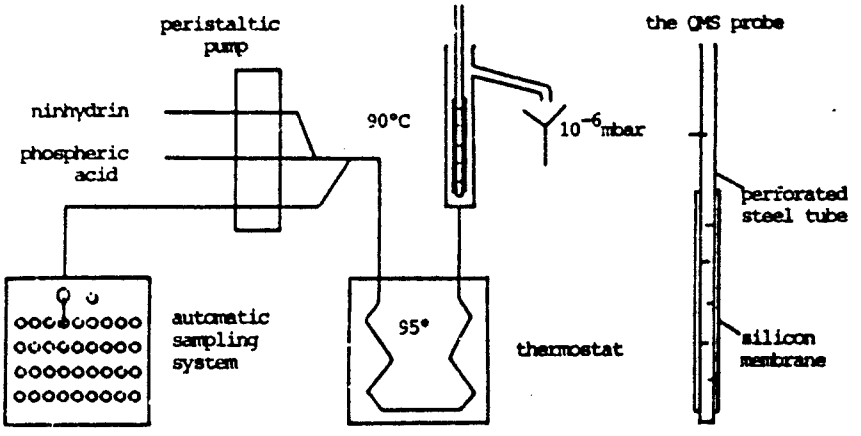


Figure 5. Determination of amino acids by mass spectrometer.

create, by genetic manipulation, new chemical entities useful for therapeutic and diagnostic purposes.

Soria said that potentially exploitable domains are present in many plasma proteins having lipid, fibrin, or collagen-binding activities. The interaction of hormones and growth factors with receptors, and the elucidation of the molecular pathways for membrane traffic inside cells, will provide effector functions for selective cellular delivery. The discovery of targeting signals for intranuclear delivery of molecules inside the cells might even allow targeting of substances to the cell nucleus. Finally, it might be possible to obtain gene targeting besides drug targeting: in the near future, transvected animals will be used to develop more accurate and predictive models for human disease, with perspectives for somatic and genetic correction therapy in humans in the future, according to Soria.

Vaccine Production by Genetic-Engineering

The topic of vaccine production by genetic engineering was discussed by J.P. Lecocq (Transgene S.A., Strasbourg, France). Lecocq said that vaccines have rapidly become preferred targets of genetic engineering. A huge effort has been made in the past 10 years but it clearly became obvious that the development of such vaccines would be long and complex. Until now, with the exception of veterinary vaccines against enterotoxins, the human vaccine against hepatitis B is the only one which can be considered as a success. Nevertheless, according to Lecocq, the use of recombinant DNA technology remains the best approach to elaborate new vaccines against numerous viruses such as AIDS and parasites such as malaria. Lecocq thinks that we can expect attractive results in the near future, especially when the need is absolute.

Results obtained by Transgene in the case of rabies are, for instance, very promising and spectacular, according to Lecocq. The new vaccine is based on a recombinant vaccinia virus expressing the rabies glycoprotein. Protection of laboratory animals can be obtained following a single skin scarification; moreover, the vaccine seems to be suitable for vaccination of wild animals (the reservoir of rabies) because foxes and racoons can be efficiently immunized by oral administration. Lecocq said that the vaccinia virus is an attractive carrier system for other antigens and he and his group are also working on a vaccine against AIDS. Lecocq thinks that genetic engineering is a powerful method for dissecting the immunological response of an animal invaded by various foreign organisms.

Mapping the S1 Subunit of Pertussis Toxin

Epitope mapping of the S1 subunit of pertussis toxin by monoclonal antibodies

was reported by R. Rappuoli (Sclavo Research Center, Siena, Italy). Rappuoli and his group have obtained several monoclonal antibodies against the S1 subunit of pertussis toxin by immunizing Balb/c mice in the footpad with whole pertussis toxin. The epitopes recognized by the monoclonal antibodies (Mabs) were mapped by Western blot using several fragments of the S1 subunit produced in *E. coli* by recombinant DNA, (or DNA) techniques. Using this method Rappuoli and his group were able to determine the regions of the S1 subunit recognized by the Mabs. In several cases, the epitopes could be identified within a few amino acids. On the basis of these data, the Mabs were classified into three groups according to their properties and used for the characterization of the functional and antigenic properties of the S1 subunit.

Neutralizing Foot-and-Mouth Disease

Neutralization and protection induced by fusion proteins containing foot-and-mouth disease virus epitopes synthesized in *E coli* was discussed by M.P. Broekhuijsen (Medical Biological Laboratory of TNO, Rijswijk, the Netherlands). He said that foot-and-mouth disease virus (FMDV) causes a highly contagious disease that affects cloven-hoofed animals. Spread of the disease is controlled by slaughter and by vaccination. Despite the great benefits of present-day vaccines, there are a number of considerations that make it desirable to improve on them, according to Broekhuijsen.

The major antigenic site responsible for the immunogenic specificity of the virus map is on one of the four capsid proteins--i.e., VP1, a protein of 213 amino acid. Synthetic peptides containing amino acid sequences 141 to 160 or 200 to 213 of VP1 can induce neutralizing antibodies in guinea pigs, although much less efficient than the virus particle itself. Broekhuijsen said that this might be caused by the small size of the peptide or by less favorable, not completely uniform folding. Therefore, he considers it desirable to search for ways to convert these peptides into more efficient determinants as a well-characterized, large-protein molecule that contains one or more copies of this determinant at its surface.

One way to obtain such molecules would be to synthesize fusion proteins comprising the antigenic determinant and a large carrier protein. Broekhuijsen and his group selected as a carrier the large bacterial protein, beta-galactosidase. He said that recombinant DNA techniques are very attractive for the synthesis of fusion proteins. Therefore, plasmids were constructed that encode beta-galactosidase fusion proteins with multiple copies of the antigenic determinant 140 to 160 of FMDV. The fusion proteins were efficiently synthesized in *E. coli* bacteria transformed with these plasmids. The fusion proteins were isolated from the soluble fraction of the bacterial lysates and purified in one step by using an affinity chromatography procedure based on the substrate-binding characteristics of the β-galactosidase moiety. In this way, 5 mg of purified protein could be obtained from 1 liter of bacterial culture.

Fusion proteins containing two or four copies, but not one, of the FMDV determinant were found to be effective immunogens. One injection induced neutralizing antibodies in guinea pigs and protected these animals against an FMDV infection. Since under similar conditions, free peptide did not elicit the same antibody response, these data indicate, according to Broekhuijsen, that the immunogenicity of this FMDV peptide can be improved by presentation of the antigenic determinant as β-galactosidase fusion proteins.

Immunogenicity of Measles Virus Protein

The immunogenicity of purified measles virus fusion protein was reported by P. de Vries (National Institute of Public Health and Environmental Hygiene, Bilthoven, the Netherlands). This was a collaborative project with scientists from the Department of Molecular Cell Biology, Utrecht, the Netherlands, and the Department of Virology, Karolinska Institute, Stockholm, Sweden. De Vries said that although live measles vaccines

have proven to be highly effective, the availability of an effective and safe inactivated vaccine would still have considerable advantages. Irreversible denaturation or undefined aggregation of the F protein, induced by the inactivation procedures could account for the failure of Tween-ether- or formaldehyde-inactivated measles virus preparations to induce biologically active antibodies against the F protein. Thus, de Vries and collaborators focused their experiments on the immunogenicity of purified measles virus F protein incorporated into iscom structures, which have recently been shown to be an efficient physical form for the antigenic presentation of measles virus membrane proteins. They selected a monoclonal antibody (Mab) directed against the F protein, which was shown to be highly effective in immunoaffinity chromatography (IAC). The incorporation of the IAC-purified F protein into iscom was achieved using an adaptation of the standard procedure for the preparation of iscom. This F iscom preparation efficiently induced biologically active anti-F antibodies in monkeys, rabbits, and mice. De Vries and coworkers also showed that the F iscom preparation induced a measles virus-specific delayed-type hypersensitivity response in mice. According to de Vries, these findings together with recent observations that dogs immunized with purified F protein of canine distemper virus (a closely related member of the Paramyxoviridae family) were shown to be protected against infection make it feasible to develop a safe and effective measles subunit vaccine.

The cloning of a gene encoding the fusion factor of measles virus was reported by S.A. Langeveld (Institute for Molecular Biology and Medical Biotechnology, University of Utrecht, the Netherlands). Measles is a childhood disease which is caused by a member of the paramyxo virus group. The measles virus is an enveloped virus with a single-stranded RNA genome of negative polarity. The genome encodes four structural proteins, one nonstructural protein, and two glycoproteins, the fusion factor (F), and the hemaglutinin protein (HA). These proteins are exposed at the surface of the viral envelope and are considered to be the major viral antigens to which the immune response is directed, according to Langeveld.

As a first step for the development of a new subunit vaccine against measles virus, Langeveld and coworkers carried out studies involving the cloning and expression of the viral genes encoding F and HA. A cDNA bank, which was constructed from poly A RNA isolated from measles-infected VERO cells, functions as a source of viral gene copies. Several specific clones were selected from this cDNA bank using nucleic acid probes. The clones were partially analyzed by nucleotide sequencing. The largest F-specific clones most likely encompass the complete gene because the translated amino acid sequence indicated the presence of a signal peptide sequence downstream from the first ATG and a proteolytic activation site which divides the F protein into a 12 kD polypeptide and a 46 kD F1 polypeptide. Langeveld said that constructions are being made to express the cDNA F gene *in vitro* and *in vivo*.

Mapping the Epstein-Barr Virus. Epitope mapping of the Epstein-Barr virus major capsid protein was reported by J.M. Middeldorp (Diagnostics Research Laboratories, Scientific Development Group, Organon International, Oss, the Netherlands). According to Middeldorp, serodiagnosis of Epstein-Barr virus (EBV) infections still largely depends upon laborious immunofluorescence techniques. Progress to more simplified methodology is hampered by the lack of a suitable cell culture system for the production of EBV-antigens. The availability of defined synthetic EBV-antigens may provide a solution to this problem.

The EBV-virus capsid antigen complex (VCA) is important for EBV-specific diagnosis; for example, detection of IgM, IgG, and IgA anti-VCA antibodies. A major antigenic component of VCA is the major capsid protein, encoded by the BcLF1-reading frame of the viral genome.

Middeldorp and his group carried out studies mapping antigenic epitopes on the EBV-BcLF1 protein as recognized by human

sera, using synthetic peptide and recombinant DNA approaches. Ten peptide regions were identified that reacted with at least seven out of ten different sera, and were compared with computer-predicted profiles of secondary structure, hydrophobicity, and with antigenic regions. Selected peptides are presently being synthesized in greater amounts for further studies, according to Middeldorp.

The production of human monoclonal antibodies was discussed by E.A.M. Stricker (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service and Laboratory for Experimental and Clinical Immunology, University of Amsterdam, the Netherlands). Stricker said that human monoclonal antibodies (MCA) can be obtained by two different procedures; (1) fusion of human B lymphocytes with a continuous cell line and (2) transformation of human B lymphocytes with the Epstein-Barr virus (EBV).

Until now, no human cell lines have been available for the efficient construction of human hybridomas analogous with the murine system. Alternatively, murine cell lines, or human-mouse xenohybrids can be used as fusion partners, but the resulting hybridomas tend to be unstable, according to Stricker. However, human B lymphocytes can be readily immortalized by EBV, which results in the continuous growth of antibody-producing cells. However, EBV-transformed cells are very difficult to clone, which hampers the isolation of monoclonal cell cultures.

Stricker and his group have produced human monoclonal antibodies against Tetanus Toxoid (TT), Hepatitis B Surface antigen (HBs) and Rhesus D antigen (RhD) as follows: PBL from donors with high serum antibody titers against the respective antigens were depleted of T-cells by E-rosette sedimentation. The non-T-cells were infected with EBV (strain B95/8), and cultured at relatively high cell numbers. After expansion, a series of low-density cultures were performed, in some instances leading to stable clones. However, proper limiting dilutions could only be performed after fusion of the EBV-transformed cells with the murine cell line NSI. The latter yielded better fusions than other HAT-sensitive murine or human-mouse xenohybrid lines. Although most EBV-transformed cells and xenohybrids cease to produce antibody, it is possible to obtain stable clones by prolonged subculture and selection, according to Stricker. Thus, Stricker and his group have obtained three anti-TT, three anti-HBs and two anti-RhD-producing cell lines. All lines produce IgG-class antibodies, except for one IgM-producer. One anti-TT antibody is biologically active in a mouse-protection assay with tetanus toxin. The anti-HBs antibodies detect all subtypes of HBs and are useful for diagnostic tests, according to Stricker.

4 CONCLUSION

It has only been possible to present selected topics from the vast array of material presented at the Fourth European Congress on Biotechnology without writing a book on all the topics presented. Biotechnology research in Europe has expanded tremendously when one considers the fairly limited amount of research presented at the Third European Congress held in Munich, West Germany, in 1984 (see ONRL Report, C-1-85). It is evident from this report on the 1987 Congress that European scientists are in the forefront of research in biotechnology.