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Review

Biosensors for process monitoring

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SUMMARY

A short review about the biosensor research activities for bioprocess monitoring in the F.R.G. after its reunification is given. The principles of biosensor applications are presented. In situ sensors and sensors based on the principles of flow injection analysis are studied. Some applications of a four-channel enzyme thermistor, bio-field effect transistors, and immunoanalysis systems for real process monitoring are presented.

INTRODUCTION

Bioprocess analysis is becoming more and more important for a better understanding and optimization of bioprocesses as well as for process safety and production documentation. Since bioprocesses involve a biological system surrounded by chemical and physical environments and the interaction between these three parts is very complex, efficient monitoring requires very detailed analysis.

Until recently, the monitoring of the chemical environment (all substrates, products and intermediates) has been performed mainly in off-line assays, which are time-consuming and often rather complex. Thus great expectations have been placed on biosensors for on-line bioprocess monitoring. By combining a highly selective biological compound with various types of transducers, biosensors provide the ability to analyze single analytes in the very complex fermentation medium in a highly selective manner. They provide a detailed insight into the bioprocess and provide the ability to fully use the potential inherent in the biological system by documenting the complex interrelation between the biological system and its environments.

BIOSENSOR SYSTEMS FOR BIOPROCESS MONITORING IN THE F.R.G.

As can be seen from Fig. 1, several research groups within the F.R.G. are involved in bioprocess monitoring via biosensor systems [1–3]. Some examples of such detailed monitoring are given in Table 1. Biosensors combine a highly specific biological compound with various types of transducers (Fig. 2). The biological compound is immobilized on the surface of the transducer or close to

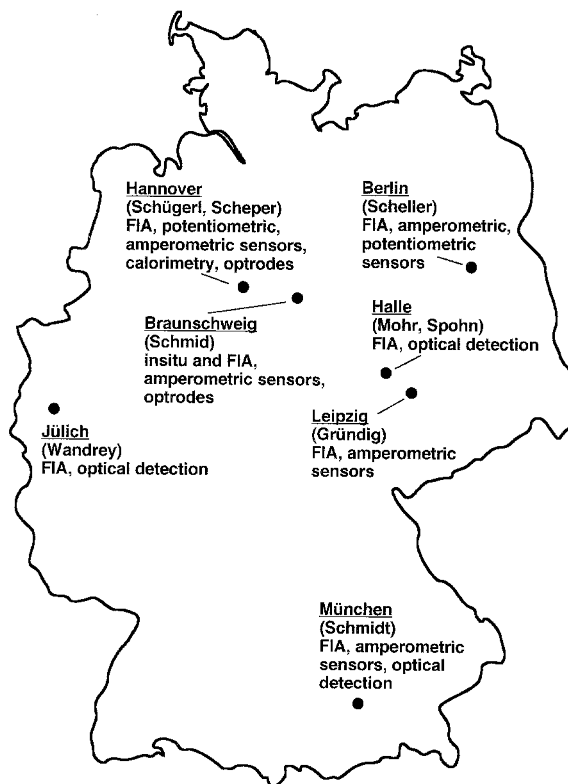

Fig. 1. German research groups involved in the development of biosensor systems for bioprocess monitoring.

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TABLE 1

Different analytes monitored via bioanalysis systems by various German research groups

Analyte	Bioprocess	Sensor system	Duration of on-line application	Reference
low molecular weight components:				
Glucose	*Saccharomyces cerevisiae*	FIA, BioFETs	30–80 h	5, 6
		in-situ electrode	12–100 h	7–9
		FIA, fluorometer	3 h	10
			4 h	11
	Cephalosporium acremonium	enzyme thermistor	160–200 h	12–14
	Cephalosporium acremonium	FIA, O_2 electrode	32 h	15
	Escherichia coli	FIA, BioFETs	30 h	6
	Corynebact. glut.	FIA, fluorometer	17 h	16
	Bacillus licheniformis	enzyme thermistor	35 h	14, 17
	Acetobacter methanolicus	FIA, amperometric	3000 assays	4
	BHK cells	FIA, optrode	220 h	18
		FIA, luminometer	220 h	19
		FIA, H_2O_2 electrode	270 h	20, 21
Maltose	*Bacillus licheniformis*	enzyme thermistor	35 h	14, 17
Sucrose	*Bacillus licheniformis*	enzyme thermistor	35 h	14, 17
	Saccharomyces cerevisiae	FIA, amperometric	1000 assays	4
Lactate	BHK cells	FIA, optrode	220 h	18
	Hybridoma cells	FIA, H_2O_2 electrode	270 h	20
	Kefir production	FIA, optrode	13 h	21
	C. thermosulfurogenes	FIA, fluorometer	–	22
Ethanol	*Saccharomyces cerevisiae*	FIA, H_2O_2 electrode	3.5 h	23
		FIA, enzyme/co-enzyme optrode	8 h	13, 24
		FIA, fluorometer	4 h	11
	Zymomonas mobilis	FIA, fluorometer	7 h	25
	C. thermosulfurogenes	FIA, fluorometer	–	22
Glutamine	Hybridoma cells	FIA, H_2O_2 electrode	270 h	20
D- and L-hydroxybutyric acid	*Corynebact. glut.*	FIA, fluorometer	17 h	16
Isoleucine	*Corynebact. glut.*	FIA, fluorometer	17 h	16
L-Leucine	*Corynebact. glut.*	FIA, fluorometer	–	11
Urea	*Cephalosporium acremonium*	FIA, BioFETs	25 h	5
	Saccharomyces cerevisiae	FIA, BioFETs	20 h	5
high molecular weight components:				
Mouse IgG	Hybridoma cells	FIA, affinity chromatography	160 h	26
		FIA, immunoassay	500 h	27, 30
Antithrombin III	Hybridoma cells	FIA, immunoassay	35 h	13, 29, 30
Pullulanase	*C. thermosulfurogenes*	FIA, immunoassay	250 h	14, 28, 30

it. Analytes react with this compound and physical or chemical properties are changed during this reaction (Fig. 2). These changes are monitored via the transducer. The total change correlates with the analyte concentration. Since biosensors cannot be sterilized without destruction of the biological compounds, in situ versions of such sensors are rather complex because the biological compound must be brought to the transducer after sterilization. Most often, systems based on the principles of flow injection analysis (FIA) are used. This technique offers tremendous advantages and its principles are shown in Fig. 3.

In this example, a continuous cell-free sample stream is withdrawn from the bioreactor either via an in situ sampling probe (e.g., ABC, München, F.R.G.) or a sampling device installed in a loop to the bioreactor (e.g.,

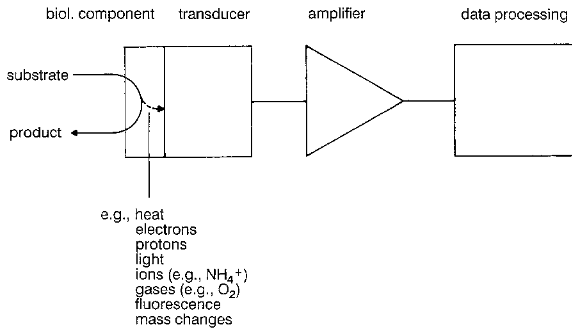

Fig. 2. Principles of biosensors.

Biopem, B. Braun Diessel Biotech, Melsungen, F.R.G.). This sampling probe forms a sterile barrier between analysis and bioprocess. The cell-free sample stream is pumped to the FIA system (e.g., the EVA system from Eppendorf, Hamburg, F.R.G.). It is feasible to condition the sample in order to match the optimal assay conditions. For example, the pH can be adjusted, the sample can be diluted, or additives can be added for a better analysis. After conditioning, the sample passes an injection valve. Under the control of a master computer, a defined aliquot of the sample can be injected into the buffer carrier stream which transports the sample to the immobilized biological compound (e.g., enzymes). This compound reacts with the analytes and the reaction is monitored via the appropriate sensor. Biological compound and sensor must not be separated as shown in Fig. 3, but can also be directly bound to each other. Data storage and processing are necessary to provide analyte concentrations and to control the whole analytical system, especially calibration and recalibration cycles. The positive attributes of this type of system can be summarized as follows:

- sample conditioning is possible;
- the system can be calibrated at any time;
- a sterile barrier between bioreactor and analysis is available;
- exchange of parts of the analytical system is possible at any time, yielding long lifetimes for operation;
- small sample volumes are needed;
- short detection times; and
- several analytes can be monitored in the sample stream.

BIOSENSOR SYSTEM APPLICATION TO BIOPROCESS MONITORING

As can be seen from Table 1, several analytes have been monitored by German research groups in different bioprocesses. Here, some applications from research projects at our institute are presented to illustrate real on-line monitoring of bioprocesses for industrial purposes. Not only low molecular weight components like different sugars and ethanol but also high molecular weight components like enzymes, antibodies, and pharmaceutical proteins were of interest. These examples should show that biosensor system application can provide very detailed insights into bioprocesses for better understanding and optimization.

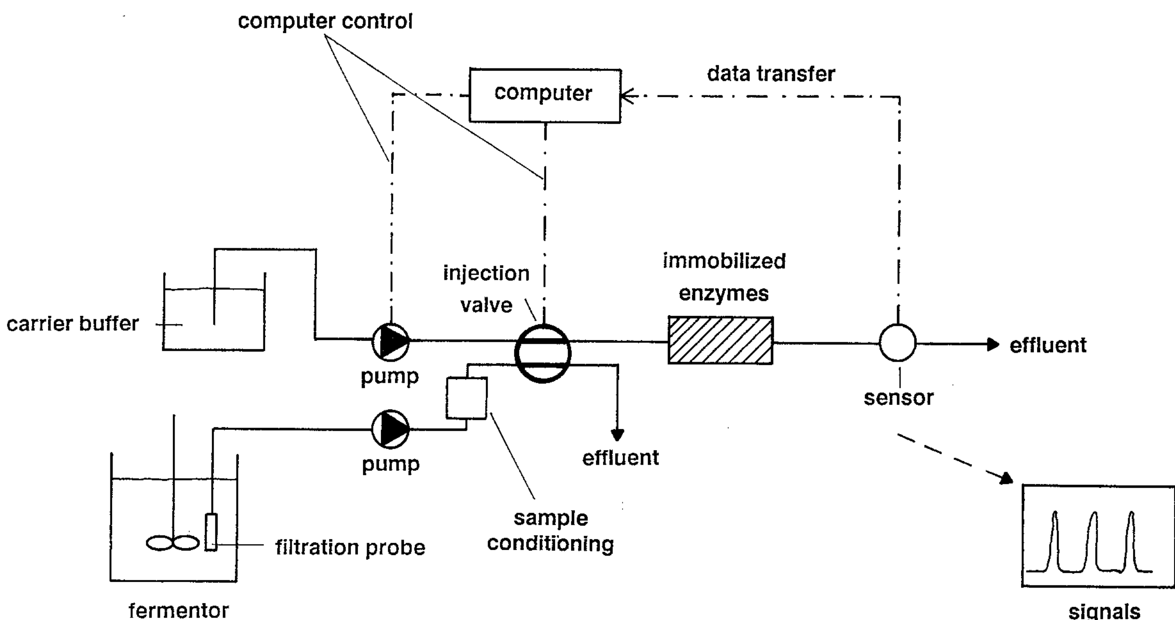

Fig. 3. Principles of bio-flow injection analysis.

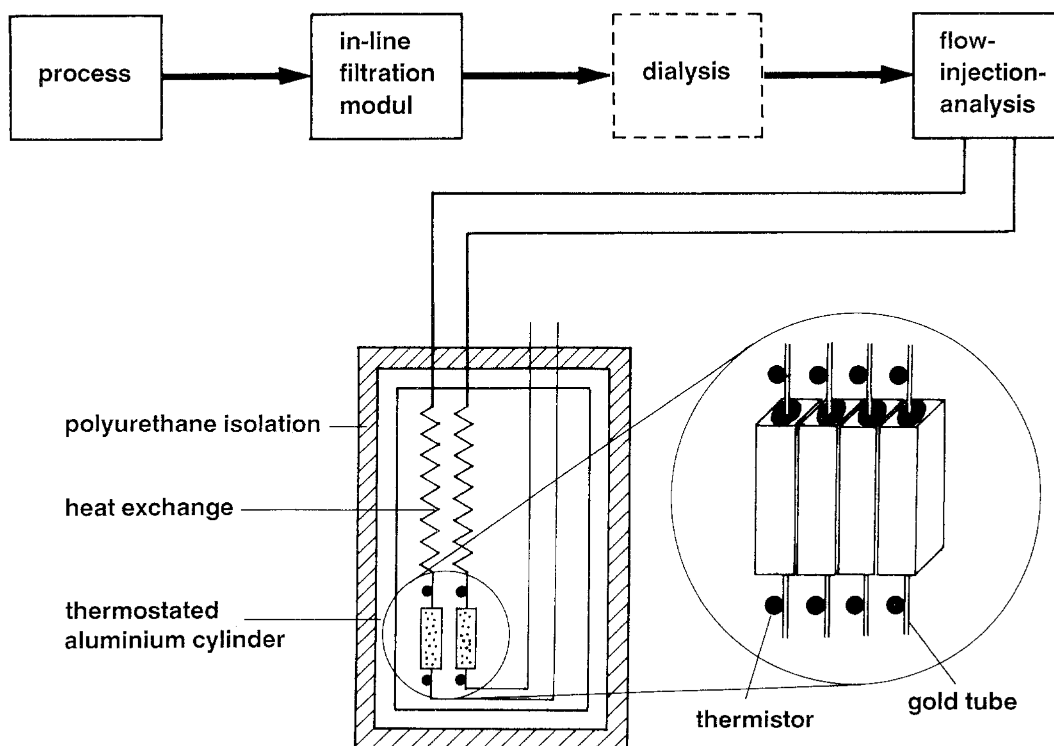

Fig. 4. The four-channel enzyme thermistor.

Enzyme thermistor

A four-channel enzyme thermistor was developed on the basis of the experiences with a Lund-type enzyme thermistor [31, 32]. With this biosensor, the heat produced during an enzymatic reaction is measured in a very well thermostated system. Using the principles of flow injection analysis, a buffer stream flows through a thermostated aluminium block (Fig. 4) where the enzymes — covalently immobilized on polymer resins — are placed in small, flow-through columns. Samples are injected into the buffer flow and are pumped through the enzyme columns, where the analyte reacts with the enzymes and heat is generated. The heat generation causes a temperature change which is monitored by small thermistors placed at the outlet of the enzyme columns. Since the whole enzyme thermistor contains four independent measuring channels, up to four different analytes can be measured simultaneously as shown in Table 2. The complete system is described in detail elsewhere [14, 17]. The system was applied to the monitoring of *Bacillus licheniformis* cultivations on technical media, and three sugars — maltose, sucrose and glucose — were detected simultaneously (Fig. 5). A sample could be analyzed every 5 min. It is apparent that one substrate after the other was metabolized by the bacteria. These results give a detailed insight into the metabolic activity of the organisms during the cultivation process. The rapid and accurate analysis is extremely important for this process because substrate concentration and protease production rate are closely connected (Fig. 6). When the sucrose concentration was high, only biomass was produced, but when the substrate level was below a critical value, both the cell activity and the productivity decreased. Based on these data, the substrate level can be accurately controlled via the biosensor system to achieve optimal protease production rates.

The accuracy of the monitoring is demonstrated for the glucose analysis during a different *B. licheniformis* cultivation (Fig. 7). Here the enzyme thermistor data were compared to off-line spectrophotometric enzyme assays

TABLE 2

Assay parameters for the simultaneous detection of different sugars

Simultaneous sugar analysis Analyte	Enzyme	Detection range	Analysis time
Glucose	Glucose oxidase	0.05–4 g/l	<3 min
Lactose	β-Galactosidase	0.05–2 g/l	<2 min
Maltose	α-Glucosidase	0.2–40 g/l	<2 min
Sucrose	Invertase	0.1–25 g/l	<3 min

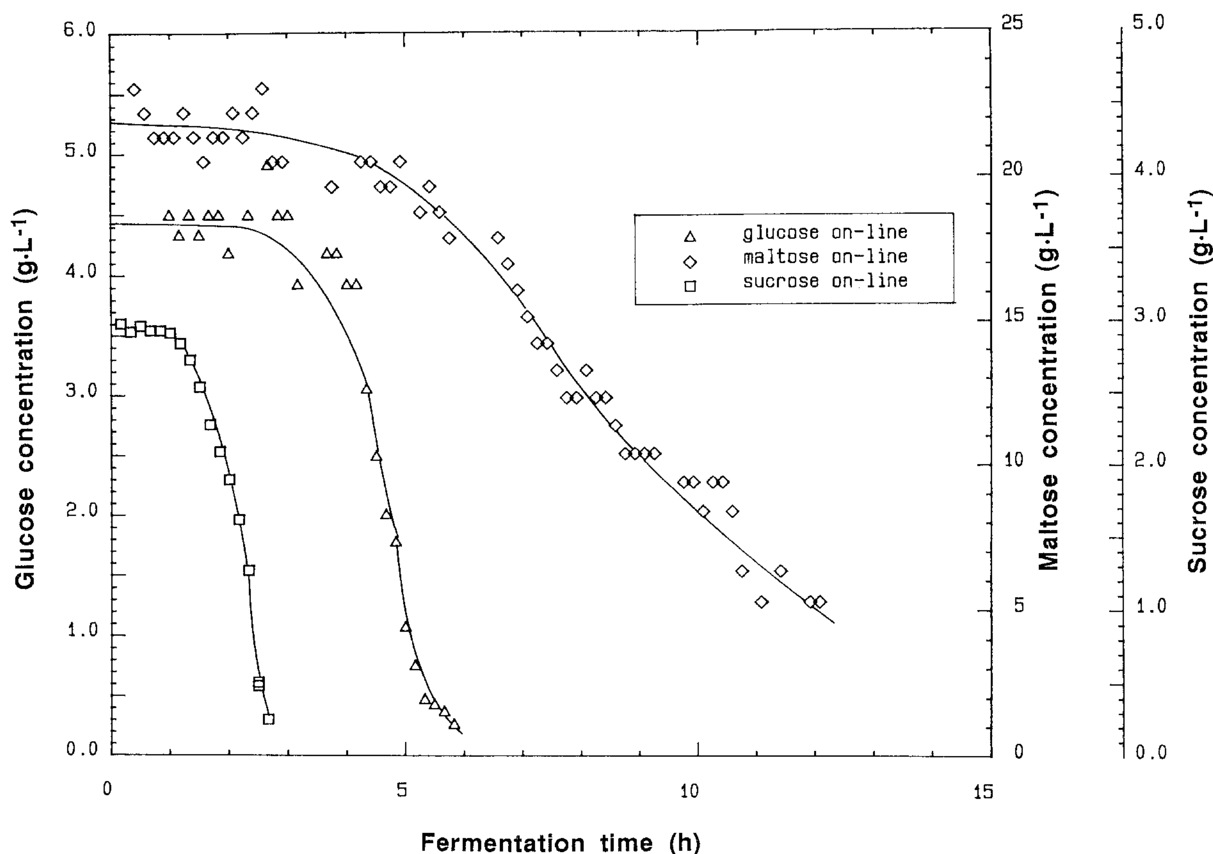

Fig. 5. Simultaneous monitoring of maltose, sucrose, and glucose during a batch cultivation of *Bacillus licheniformis*.

and off-line assays performed with a Yellow Springs glucose analyzer. The good correlation during the whole cultivation is obvious (deviations below 4%).

Bio-field effect transistors

Bio-field effect transistors are a combination of enzyme technology and modern microelectronics (Fig. 8) [5, 6, 14]. Hydroxyl groups on the surface of the gate material of the field-effect transistor extend into the aqueous solution and can be protonated or deprotonated. The ratio of the protonated to deprotonated groups affects the electrochemical potential, inducing a current between source and drain in the field-effect transistor. Thus, the pH can be measured as with a glass pH electrode.

Enzymes can be immobilized on top of the gate with the well known glutaraldehyde method [5, 6]. When a pH change occurs during the enzymatic reaction this change can be monitored via the pH-sensitive field-effect transistor (FET). The application of a glucose FET with immobilized glucose oxidase and gluconolactonase for monitoring an *E. coli* batch cultivation is shown in Fig. 9 [14]. The analysis was again performed using the principles of flow-injection analysis. A sample could be analyzed every 5 min. The correlation with off-line HPLC analysis showed a good agreement.

Immunoanalysis systems

Often, the detection of high molecular weight components like pharmaceutical proteins or antibodies are of interest in cultivation processes, especially for animal cell cultivations. These proteins must be detected in a medium among a huge variety of other proteins. Thus, immunoanalysis systems are preferable for such purposes. Since the interesting protein concentrations are high (in the range of 1 to 1000 mg/l) turbidimetric assays can be used in which no sample dilution is necessary for this concentration range [27–30]. Also, heterogeneous systems using immobilized antibodies have been reported [30]. The principles of such a turbidimetric assay are shown in Fig. 10. A sample with the antigens to be analyzed is injected into a buffer stream at the same time as a defined amount of antibodies. Both volumes are mixed and pumped through a thermostated reaction coil. Here, immunocomplexes are formed within 90 s and the turbidity of the sample is monitored continuously in a spectrophotometer. The turbidity is a measure of the degree

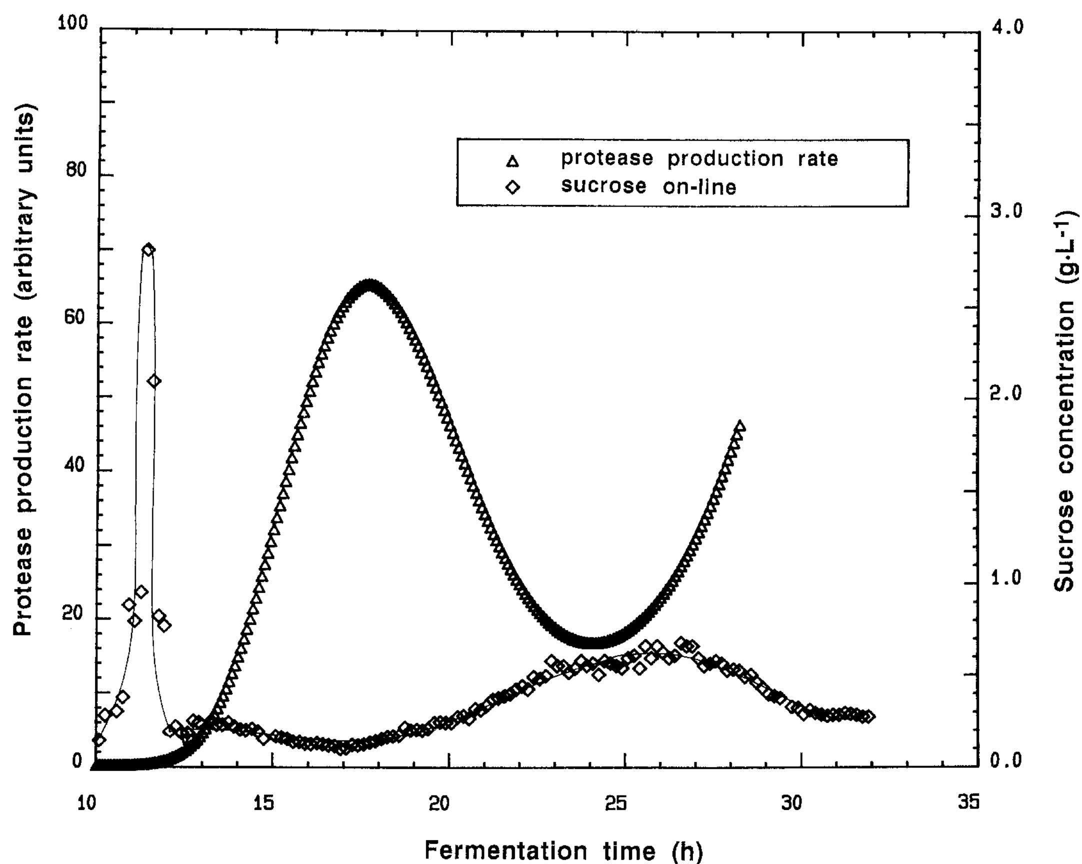

Fig. 6. Correlation between substrate concentration and protease production rate [14]. The protease production rates at different sucrose concentrations are given.

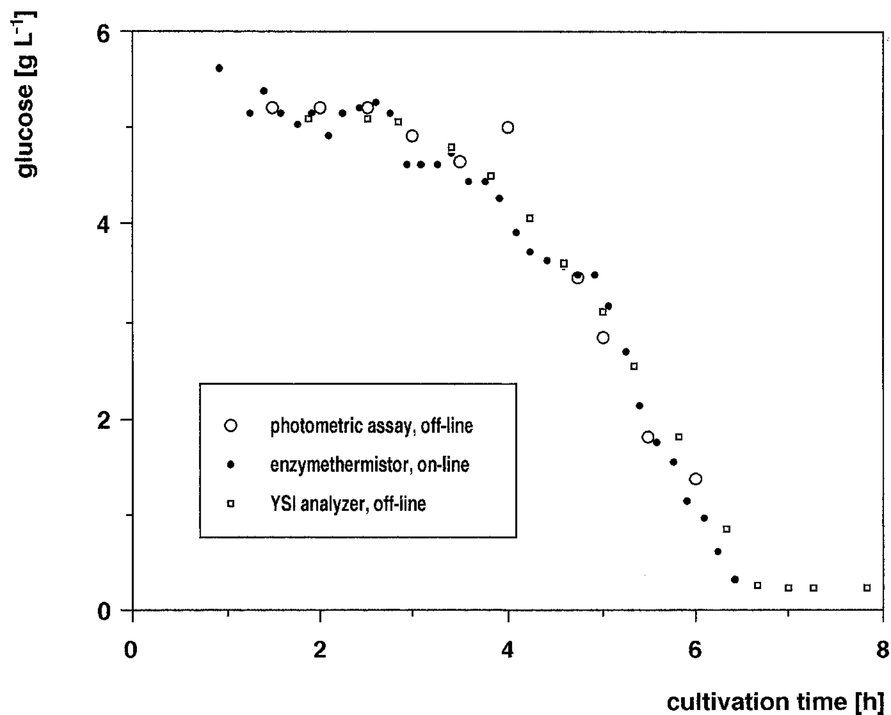

Fig. 7. Comparison between on-line and off-line data during cultivation of *Bacillus licheniformis* (deviations between 2–5%).

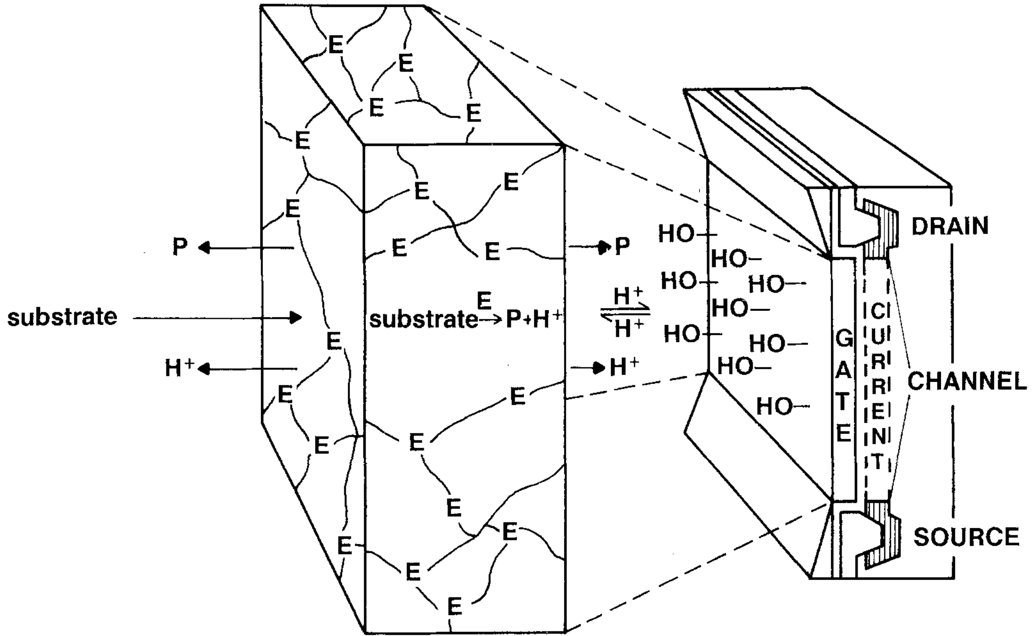

Fig. 8. Principles of a bio-field effect transistor.

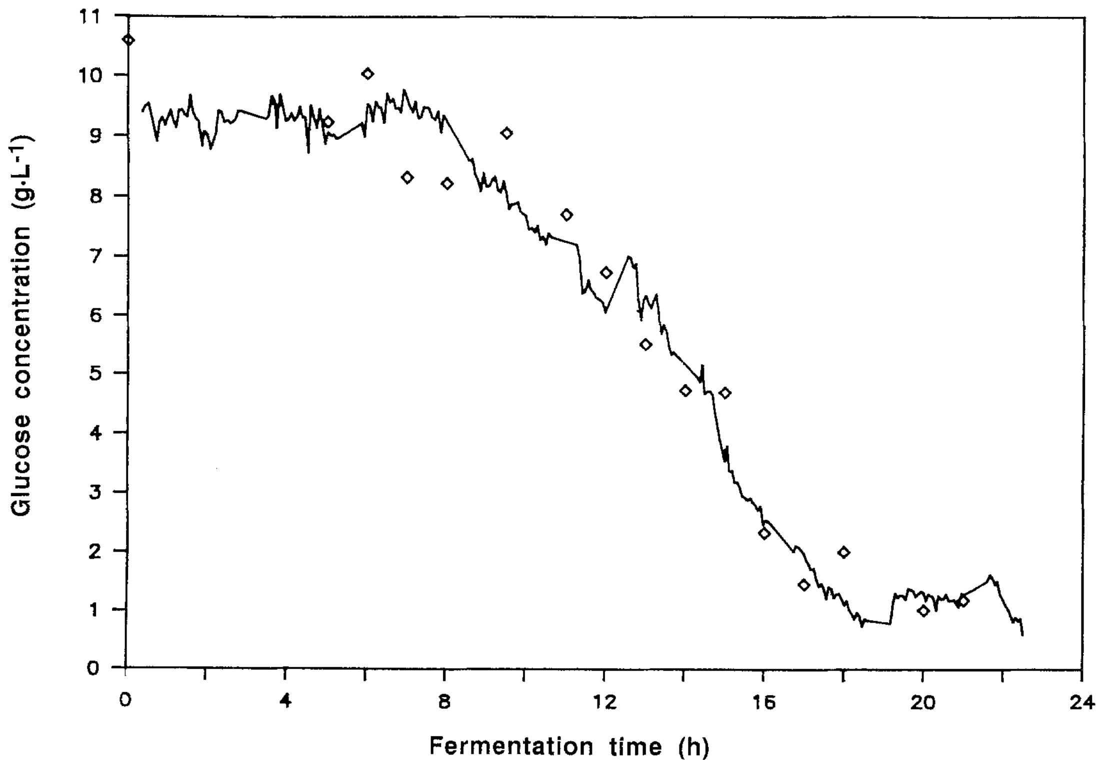

Fig. 9. Glucose concentration monitored during batch cultivation of *E. coli* (——: on-line data; ◇: off-line data) [14].

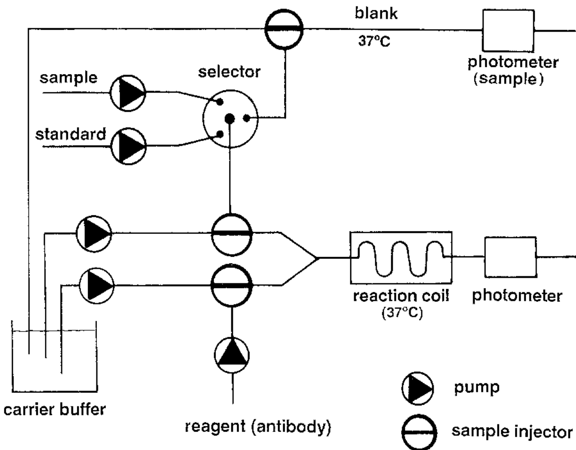

Fig. 10. Flow injection analysis system for turbidimetric immunoanalysis. The upper stream is a reference stream to check the medium absorbance.

of immunocomplex formation and is thus also a measure of the antigen concentration. A sample can be measured every 2–3 min with this type of system.

The application of the immunoassay system to a hybridoma cell cultivation is shown in Fig. 11. During the

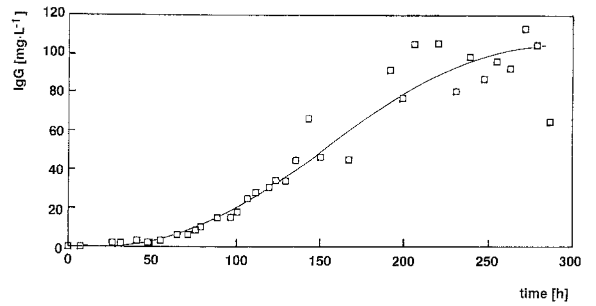

Fig. 11. On-line analysis of mouse IgG production during cultivation of a hybridoma cell [29].

first 150 h, the IgG concentration increased smoothly. In the second phase of the perfusion experiment, difficulties with clogging and reflushing of the perfusion membrane caused problems as can be seen from the drastic variations in the IgG content [27]. Another application is presented in Fig. 12. Here, the cultivation process of *Clostridium thermosulfurogenes* for the production of a thermostable pullulanase was monitored over a period of 250 h. The comparison between the on-line data and off-line data obtained in a time-consuming enzyme assay showed a good correlation over the whole bioprocess.

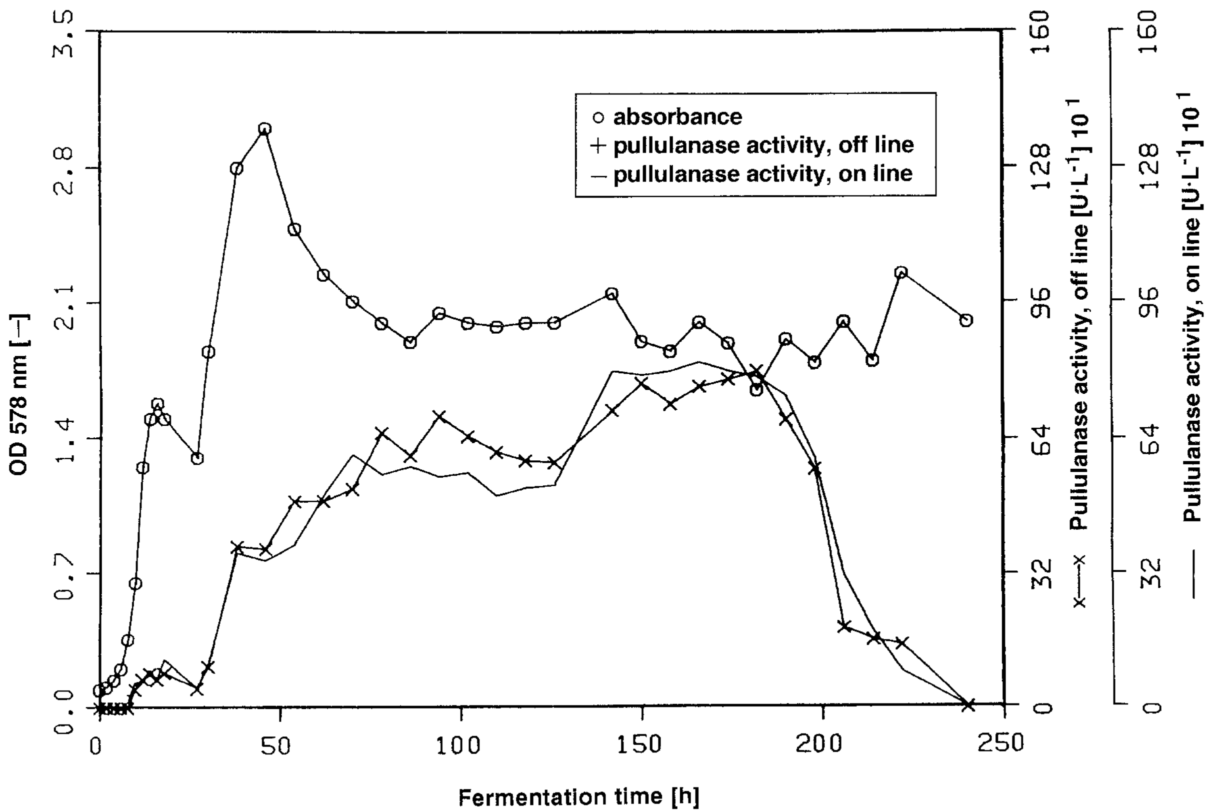

Fig. 12. Continuous monitoring of pullulanase production during cultivation of *C. thermosulfurogenes* [28].

CONCLUSIONS

On-line biosensor systems provide an impressive level of insight into the chemical environment of cultivation processes. Detailed information about the bioprocess can be obtained and this is helpful for understanding, controlling and optimizing these processes. However, biosensors have to be adapted to the bioprocess under real cultivation conditions to yield reliable results. The biosensor systems must demonstrate their reliability in comparison with conventional off-line assays. As for the conventional methods, appropriate sampling and sample conditioning is necessary, and the sample must represent the real status of the medium composition in the cultivation process.

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