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Review

Biosensors in bioprocess monitoring

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Abstract

In this review the application of biosensor systems for bioprocess monitoring are presented. Three different systems are described and applications are given: a four-channel enzyme thermistor, a three channel fiber optical biosensor and an immunoanalysis system. Biosensors can be used for bioprocess monitoring, however, the whole analytical system must include sampling, handling and sensing units.

Keywords: Biosensors; Process monitoring; Reviews

Contents

1. Introduction

Biotechnological processes are rather complex systems especially for an effective bioprocess monitoring. In these processes the biological system (e.g., cells or enzymes) is surrounded by a chemical and physical environment. Changes in one of these environments sometimes cause drastic effects on the other parts of the whole system. The aim of bioprocess analysis is a detailed monitoring of the biological system, the chemical and physical environment.

During the last 15 years biosensors have become of special interest for bioprocess monitoring. They offer the possibility to monitor single analytes even in the extremely complex and undefined media often used in biotechnology. However, in most applications biosensor analysis was performed on off-line samples [1–8]. In order to really use the analysis data, the time lag between sampling and analysis must be short enough to use the analysis data for control purposes. Thus, in a bacterial cultivation process with short generation times, the time delay

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should be in the order of a very few minutes, in cultivation processes of the much slower growing mammalian cells, this time delay is in the range of one or two hours, while in the extremely fast enzyme technology processes, the time delay can be in the order of seconds. This time delay is a function of the sampling (response time), the sample handling, the analysis and the data processing. Thus, for effective bioprocess monitoring all these single steps must be regarded, resulting in an optimized modular analysis system for each single analytical problem.

2. Sampling

Sensors can be interfaced to a biotechnological process in different ways. As shown in Fig. 1, sensors can be used as in-situ sensors (e.g., pH electrodes, pO_2 electrodes) or can be separated from the bioprocess by a filtration unit (e.g., tangential flow units in a bypass or in situ filtration units). Different systems are on the market or were developed by several research groups [9–13]. In our project an in-situ tubular sampling probe from Eppendorf (ESIP, Eppendorf-Netheler-Hinz, Hamburg, Germany) was tested. As shown in Fig. 2, a tubular micro- or ultra-filtration (pore size 0.2–0.6 μm) membrane is used in this device for sampling. The whole probe can be interfaced to the bioreactor via standard electrode ports and a peristaltic pump is used to withdraw samples with a flow-rate of 50 μl/min to 2 ml/min. For biotechnological applications, the sterility of this filtration device is important. No microorganism is allowed to cross this barrier. In order to test the efficacy of the system, a bubble-point measurement can be performed. For this purpose, air is pumped into the system. When a critical pressure is reached, air bubbles pass the filter (bubble-point). So long as the bubble-point is above 0.5 bar, the sampling probe can be regarded as sterile for both types of membranes. If the bubble-point is below this critical pressure, the system can no longer be regarded as a sterile barrier. The membrane might be broken or the whole probe could be defective. In Fig. 3 the results of a bubble-point measurement are shown. Air was pumped into the sampling device with increasing pressure. No leakage could be observed up to 2.1 bar. At this pressure, bubbles pass the membrane and a measureable flow-rate of the gas can be observed.

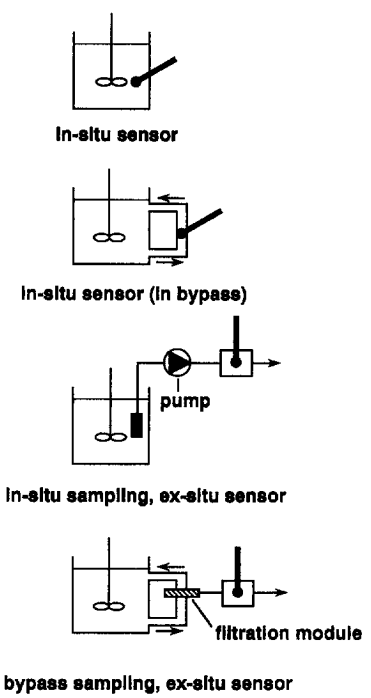

Fig. 1. Different ways to interface sensors to bioreactors.

The second important feature of sampling devices is the response time. In order to achieve a real on-line monitoring, the response time must be as short as possible. As shown in Fig. 4, the response time of a stepwise increase of the salt concentration in a bioreactor is about 5 min at flow-rates of about 1.5 ml/min [10,11]. These response times can still be obtained after several sterilizations and they can be shortened at higher flow-rates. It is necessary to provide a constant flow across the membrane; otherwise fouling might become a major problem. In order to keep the sample volume low, the flow-rate can be reduced to lower values between the analyses (e.g., 100 μl/min). This optimized sampling strategy would provide short response time at small total sampling volumes.

3. Biosensor integration

For biotechnological application, the biosensors can be integrated into a flow injection analysis

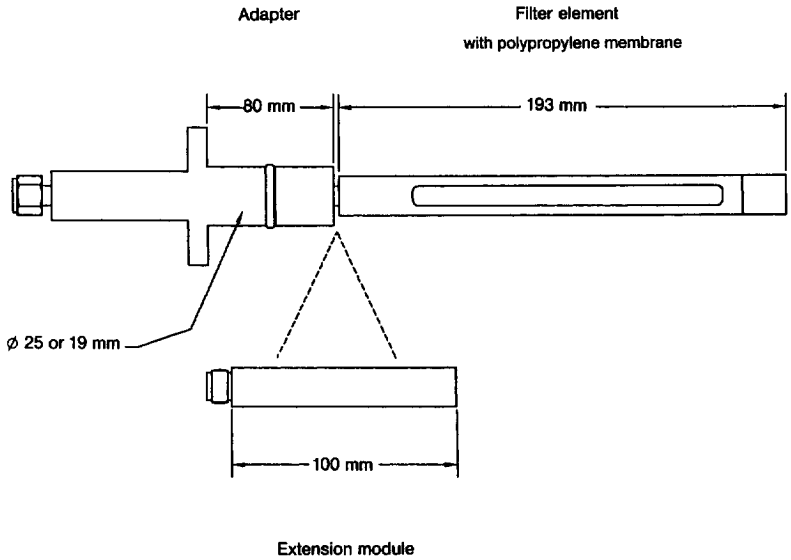

Fig. 2. Set-up of the ESIP sampling device (Eppendorf-Netheler-Hinz, Hamburg, Germany). Tubulare ultra- or micro-filtration membranes can be used in the filter element part. This part sticks into the medium. Special extension modules can be used to elongate the filter element part. The probe can be interfaced via standard electrode ports to bioreactors.

system. Aliquots from the cell-free sample derived from the sampling unit are injected into a continuously flowing buffer stream [5,14,15]. This carrier buffer stream transports the sample to the biosensor. Several advantages such as sample treatment, short contact times of the sample with the sensor and easy recalibration are inherent in these BioFIA (bio-flow-injection analysis) systems [16]. Normally, computers are used for system automatization and data processing. Thus, the whole analysis system can be used as a stand-alone version. In order to illustrate the potential of such BioFIA systems three different examples and their applications for bioprocess monitoring are given in the following sections.

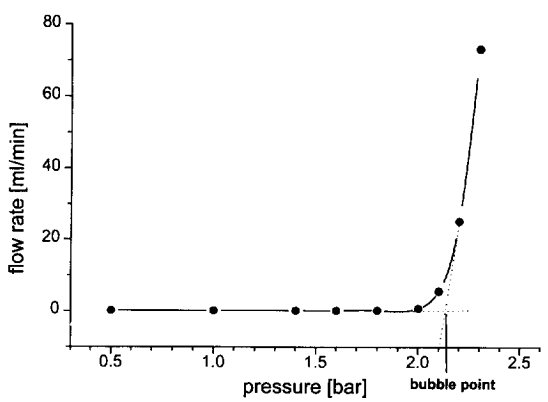

Fig. 3. Bubble-point measurement after four sterilization procedures. At pressures above 2.1 bar, gas can penetrate through the membrane, thus a flow of the gas through the membrane can be observed.

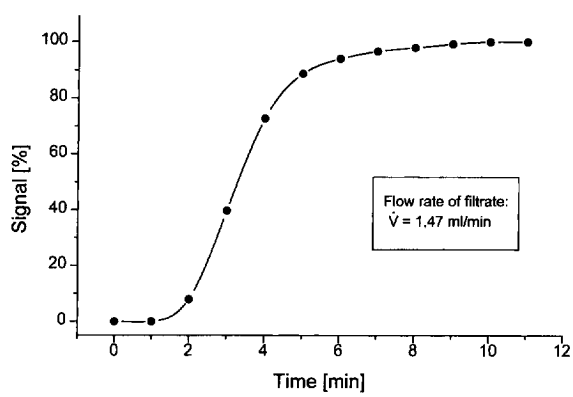

Fig. 4. Response time behaviour of the ESIP sampling device in cultivation media. A KCl pulse was added to the bioreactor and the conductivity in the filtrate was measured continuously.

4. Calorimetric sensors

Based on the fundamental work on enzyme thermistors performed at the University of Lund [17–19], a four-channel enzyme thermistor was built in our laboratory for the simultaneous detection of up to four analytes with immobilized enzymes (Fig. 5) [20]. Sucrose (immobilized enzyme: invertase), lactose (immobilized enzyme: β-galactosidase), maltose (immobilized enzyme: α-glucosidase) and glucose (immobilized enzyme: glucose oxidase/catalase) could be analyzed directly even in colored cultivation media with analysis frequencies of 20–30 samples per hour. When the sample injected came into contact with the immobilized enzymes, the enzymes reacted with the corresponding analyte. During this reaction, heat is generated, causing a temperature change which is detected by the thermistors placed at the top and the bottom of the cartridges. The application of the enzyme thermistor system to a batch cultivation of *Bacillus licheniformis* on a technical medium (hydrolyzed starch medium) is presented in Fig. 6. One substrate after the other is consumed by the organisms. In addition, the hydrolysis of disaccharides by excreted enzymes becomes obvious, because the sucrose is consumed prior to the monosaccharides. The whole system was stable over fermentation times of several days, recalibration was performed every hour. No negative effect of the proteases produced during this industrial process on the immobilized enzymes could be observed.

However, not only cultivation processes but also biotransformation processes using immobilized enzymes are of interest. The time scale of these biotransformation processes is in general short and the analyte concentrations are very high. Thus, biosensor analysis must be performed at very high frequencies and the range of applications regarding the analyte concentration should be broad. Again, enzyme thermistors are ideal process-monitoring devices.

In Fig. 7 the application of the enzyme thermistor with immobilized urease is shown for the enzymatic production of L-ornithine from L-arginine by immobilized arginase. The immobilized enzyme is used in a stirred tank reactor dispersed in an aqueous arginine solution. An enzyme-free sample is recirculated through a bypass. An aliquot of this sample stream can be injected into the enzyme thermistor for analysis. As soon as the ornithine concentration reaches a certain level, the reaction is stopped by

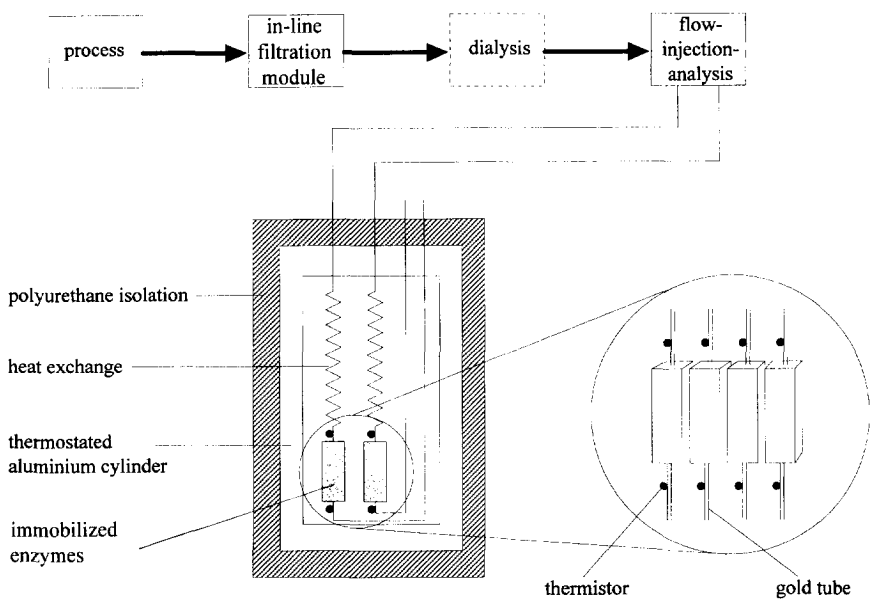

Fig. 5. Principle of the four-channel enzyme thermistor device.

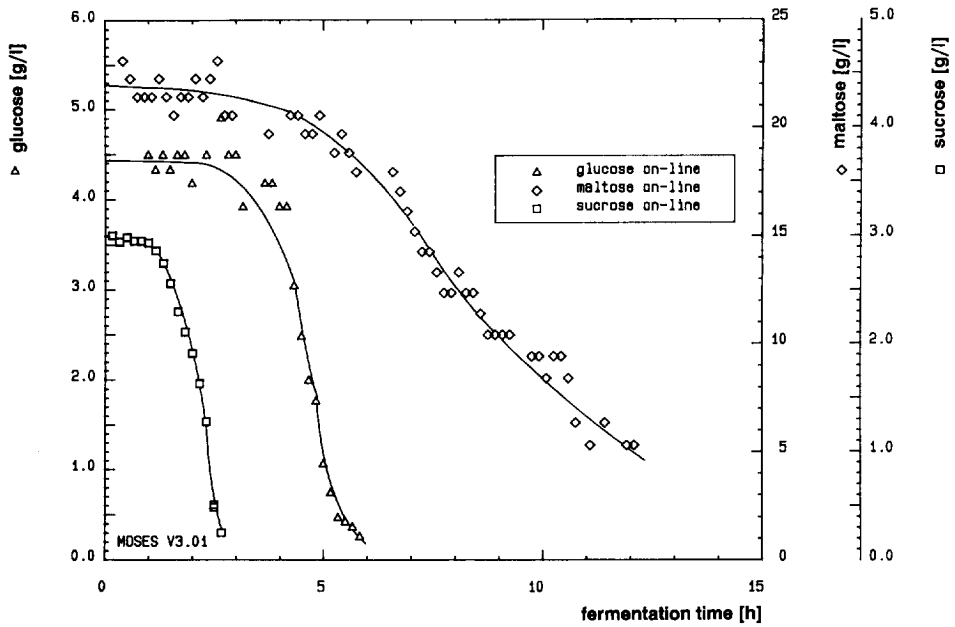

Fig. 6. Application of the four-channel enzyme thermistor for the on-line monitoring of a batch cultivation of *Bacillus licheniformis* for the production of protease.

withdrawing the whole reaction mixture and is restarted by adding fresh substrate solution into the reactor. The whole process can be controlled by the computer of the enzyme thermistor system. Short analysis times of 3–4 min per sample are adequate for this process. In the literature, more examples of the application of calorimetric systems for the analysis of biotransformation processes are given [19,21], such as the fast analysis of enantiomeric excesses even in organic samples.

5. Optical sensors

Fiber optical sensors offer tremendous advantages for bioprocess-monitoring. Optodes can easily be miniaturized and the signals can be transported via glass fibers over long distances without problems of interferences. The principle of an enzyme optode is shown in Fig. 8. The basic element is a pH-optode. Here a pH-sensitive dye is immobilized on the tip of a glass fiber [22] in a polyvinyl alcohol matrix. The excitation light is guided through the fiber and irradiates the fluorophor. The generated fluorescent light travels backward through the fiber and is detected via a photomultiplier. The fluorescence intensity is a function of the pH [23]. This pH-optode can be covered with an enzyme-containing membrane. In the example described, urease was immobilized in a glutardialdehyde membrane on top of the pH-optode [22,23]. The pH-shift in a urease containing sample caused by the enzymatic reaction is followed by a shift in the fluorescence intensity which can be correlated with the urea concentration. However, it is always a problem to know accurately if the signal change is derived from an enzymatic reaction or from a pH-change in the sample. As can be seen in Fig. 9, the influence of small pH-changes on the sensor readout is large. Smaller deviations in the pH-value of the sample cause changes in the sensor readout, as can be seen from the 3-D-plot. Thus, in order to achieve high signal reliability, it is necessary to monitor all variables that might influence the signal. Only, when the exact pH of the sample is known, can the signal change due to the pH-change caused by the enzymatic detection reaction, be used to calculate the concentration.

Based on these results, a three-channel optical fiber system was developed to monitor up to three different analytes simultaneously. The principle of

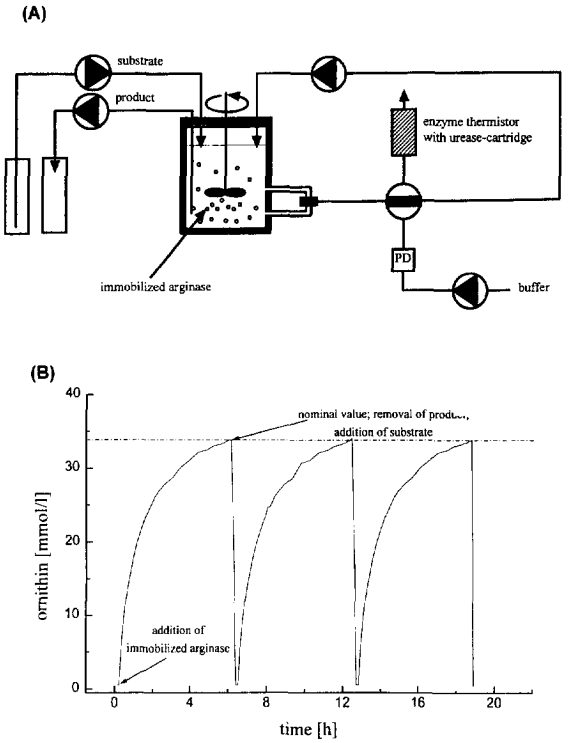

Fig. 7. Application of the enzyme thermistor for the monitoring of a biotransformation process. (A): setup of the whole biotransformation process: batch-reactor: 1000 units arginase immobilized on VA-Epoxy®-resin (Riedel-de-Häen, Seelze, Germany), 0.1 mol/l potassium phosphate buffer (KPP), pH 9.5, 40 mmol/l L-arginine as starting solution; enzyme thermistor: 250 units urease immobilized on VA-Epoxy®-resin, carrier buffer: 0.1 mol/l KPP, pH 7.0, 1 mmol/l cysteine as stabilizer; (B): Production process controlled via the enzyme thermistor. As soon as a certain concentration of the product is reached, the reaction is stopped and fresh substrate medium is added to the reactor.

the system is shown in Fig. 10. The excitation light is focussed via a dichroitic mirror onto a trifurcated fiber cable guiding the light through a chopper

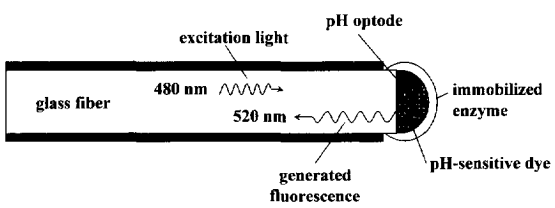

Fig. 8. Principle of a fiber optical biosensor. Here, a pH optode is used as a transducer unit.

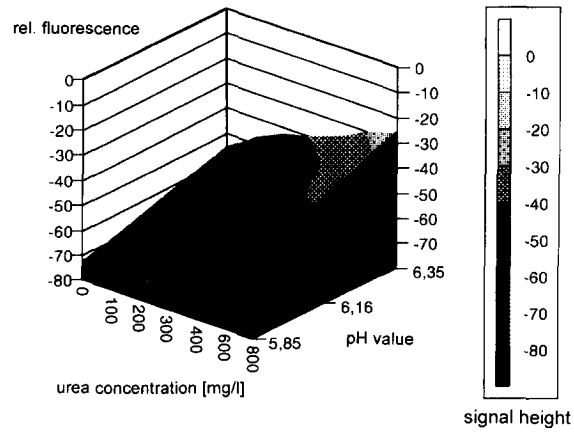

Fig. 9. Influence of the urea concentration and the sample pH on the sensor signal of the pH-dependent fiber optical biosensor. The sensor signal is influenced drastically for a given urea concentration by small changes in the sample pH.

wheel. Here, according to the number of holes in the chopper wheel for each cable, a light modulation is performed. Three optodes with a modulation of f, 2f and 3f can be used. The f optode was used as enzyme optode, while the 2f optode was used as pH optode (the 3f optode was not used for the experiments described). The signals derived from each optode have the same modulation. The fluorescent light passes the chopper wheel and the dichroitic mirror and is then detected via the photomultiplier tube. The signals can be demodulated using a lock-in amplifier, so that up to three different analytes can be monitored simultaneously.

An application of the system is shown in Fig. 11 for a hemodialysis experiment. A given urea concentration — typical for hemodialysis — was analyzed continuously with the multi-channel sensor system. Two optodes were placed directly in the effluent stream. As long as the pH was constant, an accurate sensing was possible with the signal from the enzyme optode only. After 240 min, the pH was changed slightly, followed by a completely wrong urea analysis, when only the enzyme optode is used. When the simultaneously measured pH-value is used for signal correction, an accurate analysis is still possible. Here, the calibration curve for the enzyme optode signals at the simultaneously measured pH-value are used. For this purpose calibration graphs at

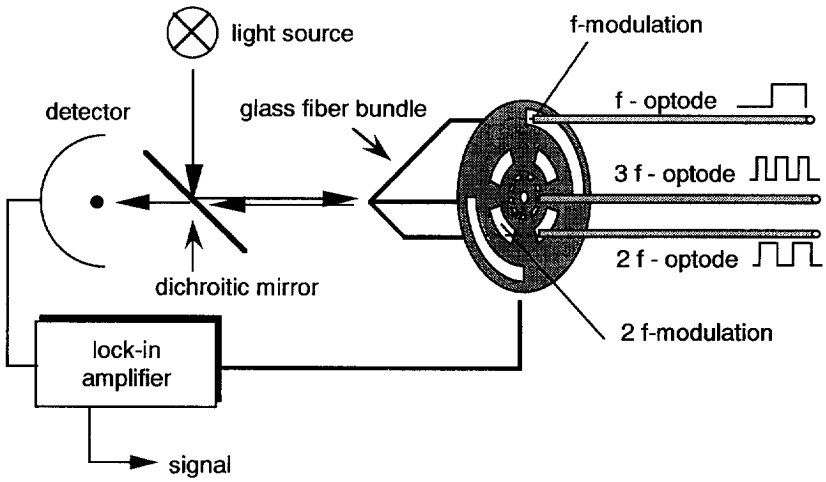

Fig. 10. Principle of the three-channel fiber optical sensor systems. Three optodes can be used to measure three analytes simultaneously.

all different pH values must have been analyzed before the experiment was started or the 3-D-data from Fig. 9 can be used in a more complicated calculation. When the sample is brought back to the original pH-value, the same effects can be observed. This whole procedure shows, that multicomponent analysis is necessary especially when sample handling is not possible [23].

6. Immunosensors

The analysis of proteins has gained tremendous importance in biotechnology. Several proteins such as blood factors and monoclonal antibodies are produced in mammalian cell cultivation processes. Here, the target proteins must be detected in media containing several other proteins. Typical concen-

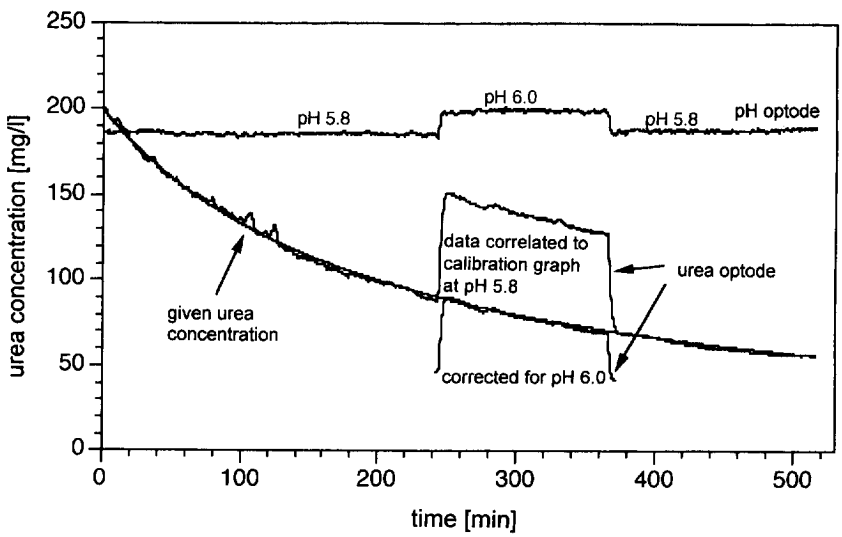

Fig. 11. Application of a two-channel optode system for monitoring of a hemodialysis experiment. The pH and the urea concentration are measured simultaneously.

trations are in the range of 1 to 1000 mg/l. Immunoanalysis techniques are the system of choice for this purpose. Normally the test plate ELISA (enzyme-linked immunosorbent assay) [24,25] techniques have been used, which are time consuming and costly. In addition, several dilution steps may be necessary to bring the actual cultivation concentrations down to the optimal detection concentrations. Thus, the analysis data are often available with a time delay of 24 h after sampling.

In order to obtain fast and reliable monitoring of the actual state of a cultivation process, an automated immunoanalysis system was set up. The principle is shown in Fig. 12. An aliquot (10–50 μl) of the protein containing sample is injected into a continuous flowing buffer stream. This sample is pumped into a cartridge which contains appropriate antibodies immobilized on a polymer resin [26]. In addition, immobilized protein G can be used for the analysis of IgGs. The target proteins bind to the immobilized affinity compound and are trapped inside the cartridge. All other proteins or medium compounds are washed out. After this binding and washing step, an elution is started. Here a shift in the pH or the ionic strength of the buffer is appropriate for an efficient elution without affecting the conformation of the affinity compound [26]. The concentration of the eluted proteins can be detected via a spectrofluorometer, measuring the inherent protein fluorescence. Up to 1000 analyses can be performed with one cartridge. For all affinity systems tested to date, pH-changes in the range of two were appropriate. No effects on the support materials were observed (polymers, Sephadex). The analysis time is in the range of 5 to 6 min. The application of this system is shown in Fig. 13. Here, antithrombin III (AT III) was analyzed alone and in the presence of bovine serum albumin (BSA). BSA was added to the sample at concentrations up to 100 times higher than the AT III concentration. Since all calibration graphs have the same slope, the separation of the target protein from interfering proteins is effective in the system. In Fig. 14 the application of the analysis system for the monitoring of the production of monoclonal antibodies in a hybridoma cell cultivation process is shown. There is good correlation with results obtained using a conventional ELISA assay. The standard deviation is in the range of 3–4% for the heterogeneous immunoassay. The main advantage of the assay is the speed and frequency of analysis. A sample can be analyzed within 5 min without further treatment, giving the chance of direct bioprocess monitoring.

7. Conclusions

Analysis for bioprocess monitoring must fulfill different tasks. The analysis systems must work as stand alone systems with high analysis frequencies in order to make a direct process analysis possible. However, the whole bioprocess analysis must be

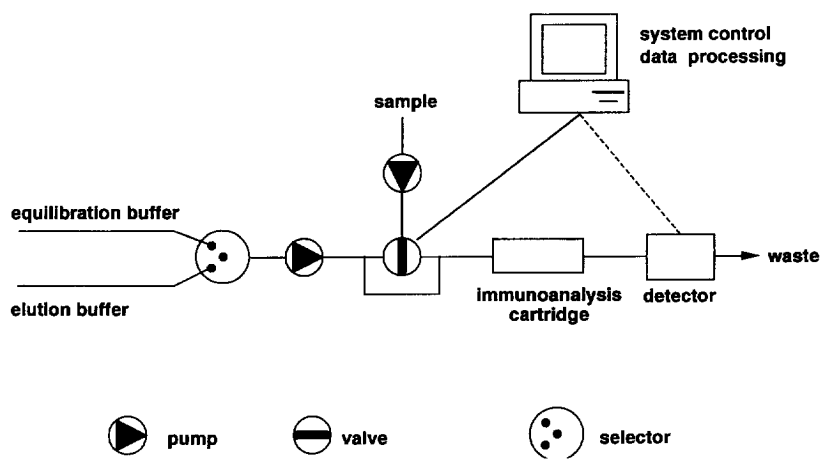

Fig. 12. Principle of the automated heterogeneous immunoanalysis system.

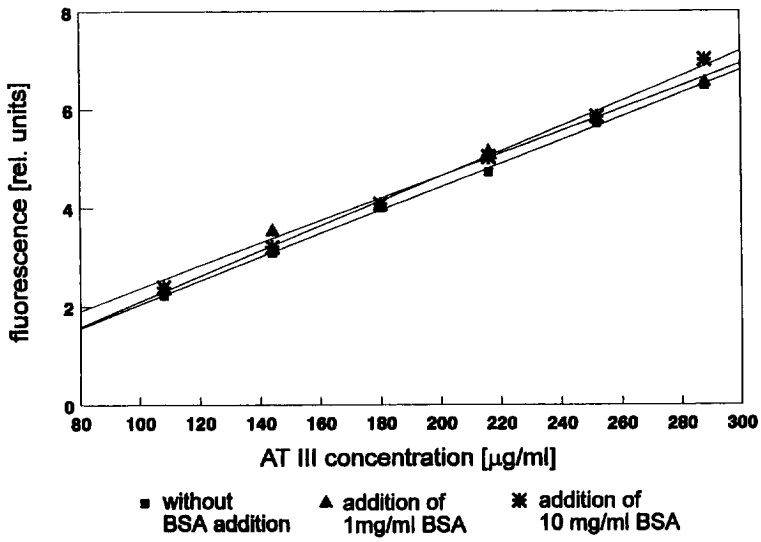

Fig. 13. Application of the automated immunanalysis system for antithrombin III (AT III) analysis with various amount of possibly interfering serum albumin in the sample. An AT III sample (in cultivation medium) was injected without and with BSA addition, respectively. Sodium phosphate buffer was used as carrier buffer (pH between 6 and 7, 100 m*M*). The elution was performed by a pH shift in the buffer to pH 2.

considered as the sum of sampling, handling and sensing of the sample from the bioreactor. If the analysis data is to be used for control purposes, the time lag between sampling and the final analysis must be shorter than the generation time of the cultivated cells. Multicomponent analysis is not only necessary to get a more detailed insight into the process but also to obtain more reliable data. Bringing all these statements together, an analysis system for bioprocess monitoring must be flexible and problem oriented. Knowledge about the process, the instrumentation and the analysis procedure must be brought together.

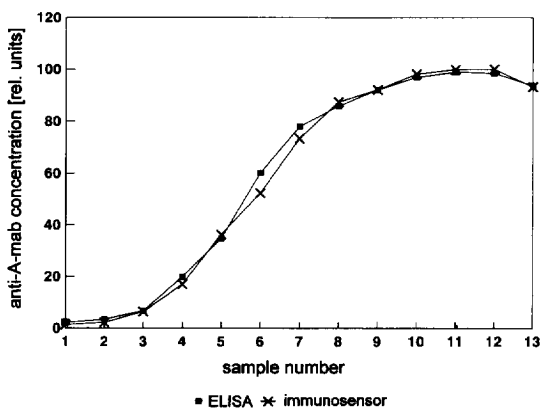

Fig. 14. Comparison of the data obtained with the conventional ELISA and the fast automated heterogeneous analysis system for the detection of a monoclonal antibody. About 50 µl sample are necessary for the heterogeneous assay and about 1 ml for the ELISA. The elution was performed as given in Fig. 13 [26].

Acknowledgements

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