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Bioanalytics: detailed insight into bioprocesses

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Abstract

The principles of bioanalytical systems for an on-line bioprocess monitoring are described within this paper. These sensor systems can be interfaced to the bioprocess in different ways according to the needs of the single bioprocess. Modular systems are necessary, which can fit exactly to the needs of the single process. Invasive as well as non-invasive bioanalytical tools are described and discussed in detail. Immunosensors give the possibility to monitor high molecular weight components within short time intervals. Non-invasive optical sensors allow the direct monitoring of various analytes such as oxygen pH for the complex fluorescence behavior of the bioprocess medium. These so-called fluorescence sensors offer the possibility to monitor intra- as well as extracellular components without interfering with the bioprocess. An industrial example for the application of bioanalytical tools for a process optimization are presented in this application. Here a biosensor system is used to optimize the downstreaming of molasses on a technical scale. The economic as well ecological advantages are discussed.

Keywords: Bioprocess; Biotechnology; Immunoassay; Biosensor; Fluorescence

1. Introduction

Biotechnology is regarded as one of the megatechnologies of the next millennium. Industry, as well as our daily life, will be tremendously influenced by this technology in the very near future. The main focuses of biotechnology are pharmaceuticals/medicine, agrochemicals, food technology and sustainable development. In general, biotechnological processes are extremely complex and cells or microorganisms must be cultivated under optimal and defined conditions with regard to viability, reproducibility and productivity. The cells themselves form the biological system in a bioprocess, which is surrounded by a physical and chemical environment (Fig. 1). The productivity, activity and metabolic state of this biological system is often extremely sensitive to environmental influences. The interaction between these three compartments of a bioreactor is rather complex. In order to drive the biochemical reaction network of the biological system in the correct direction, the complex interaction of the overall system must be understood and controlled. Therefore, detailed knowledge is required about the three compartments of a bioreactor.

In addition, bioprocesses often include a downstream processing step which is frequently the bottleneck of the overall process. Here the products must be recovered from a rather complex reaction mixture.

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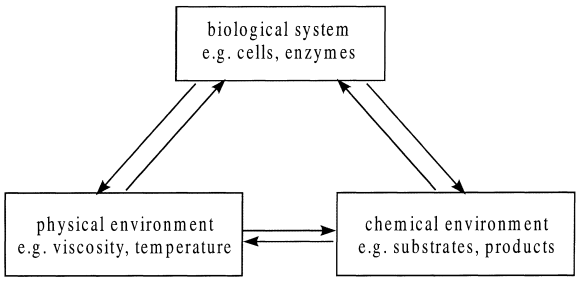

Fig. 1. Close interaction between the three compartments of a bioreactor.

Detailed monitoring of these processes is also necessary in order to optimize the recovery process with regard to quantity as well as quality (e.g. biological activity) of the products. Analytics play a key role in bioprocess understanding, optimization and quality control and tremendous efforts will be necessary to meet future ecological and legislative demands.

Analytical systems have been developed in a huge variety for different applications in the area of life science. Several applications for use in bioprocess monitoring are described and reviewed in the literature [1–6]. However, additionally new analytical systems or procedures must be developed to meet the special demands of modern and future bioprocessing or additional efforts are necessary to adapt the already existing systems for a efficient bioprocess monitoring. Since modern processes are extremely complex and vary extremely from bioprocess to bioprocess, (e.g. mammalian cell cultivation in comparison to bioremedation). Appropriate analytical tools must be developed, which can fit exactly to the needs of the bioprocess by combining different variable basic modules.

This paper describes the principles of bioprocess monitoring and a selective immunological biosensor monitoring system as well as a more integral optical monitoring system are presented. The application of these systems for the optimization of an industrial process with regard to ecology and economy are also considered.

2. Principles of bioprocess monitoring

Sensors can be interfaced to a bioprocess as in situ or ex situ sensors (Fig. 2). In situ sensors, such as pH or p_{O_2} electrodes can be directly installed in a bioreactor port and will give continuous information about the bioprocess. These sensors must be sterilizable since they are in direct contact with the bioprocess medium. Ex situ sensors are installed outside the bioreactor and a representative sample of the bioprocess is pumped via an appropriate sampling system to the analysis unit. Here cell containing samples or cell-free samples can be analyzed. Problems arise due to sterility conditions, that means the sampling unit must provide a sterile barrier between the analytical system and the bioreactor. The withdrawal of cell containing sample is easy in perfusion cultures. Otherwise complex robotic systems are needed which withdraw the cell containing sample from the bioreactor portionwise. The sample composition will change due to the metabolic activity of the cells within the time gap between sample withdrawal and analysis when cells are present in the sample. In order not to falsify the analysis the cell activity must be stopped (e.g. by adding stopping solu-

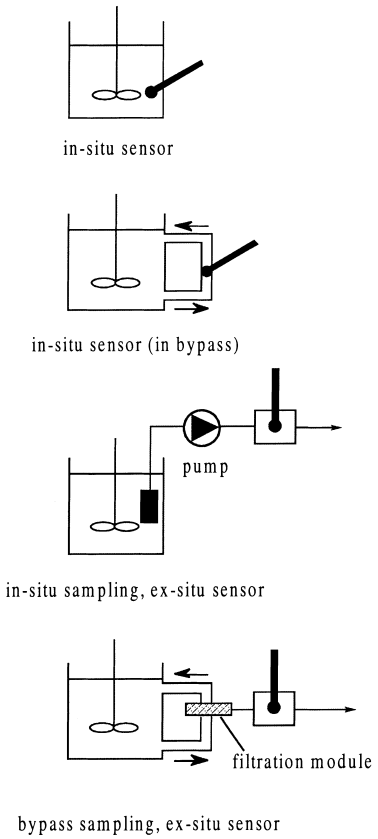

Fig. 2. In-situ and ex-situ bioanalysis.

tion). Different devices are described such as catheter probes to withdraw cell containing samples combined with the blocking of cell activity [2]. Such systems are also used to withdraw samples at extremely high frequencies in order to get information about the dynamic behavior of the biological system [8].

Special filtration devices are available which offer the possibility to withdraw cell-free samples from the bioprocess. Here the metabolic active cells are retained in the bioreactor and the composition of the sample does not change after the withdrawal. Such devices can be used in a bypass (e.g. cross-flow filtration systems) or in situ (e.g. tubular membrane probes [7]. Different devices are commercially available based on micro- or ultrafiltration, dialysis or even pervaporation principles [2].

The time gap between sampling representing the actual state of the bioprocess and the availability of the analytical data differ tremendously. The distance between sampling probe and analysis system and the overall assay time determine, if the data are available in real time or with a more or less time delay. In addition, off-line analysis systems are often interfaced to bioprocesses based on the principles of flow injection analysis. That means that the withdrawn sample is injected at different time intervals into a carrier buffer stream which transports the sample to the analysis unit. A continuous monitoring is no longer performed but a portionwise or quasi on-line monitoring with a time delay is achieved. In general it is not possible to develop an optimal bioanalytical system which can meet the needs of all different bioprocesses. However, it is possible to develop an analysis unit which fits optimal to a certain bioprocess, especially also in regard to analysis time. Fig. 3 illustrates these features which are called 'in-time analysis'. In mammalian cell cultivation changes are very slow, due to the slow cell division. If data are available in an hourly time interval, they still can be used excellently for a direct bioprocess control. However, when cell division becomes faster (e.g. *E. coli*) data must be available within minutes in order to control the process. In downstream monitoring the time intervals for an appropriate process monitoring might even become smaller since it might become necessary to distinguish between different fractions within seconds due to the throughput flow of the downstream unit. In conclusion it becomes obvious that the bioprocess itself rules the optimal time

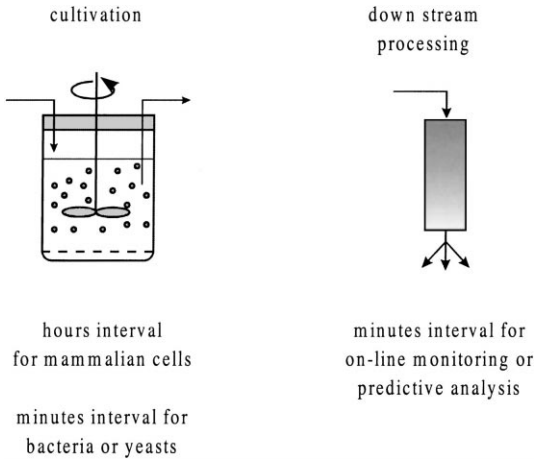

Fig. 3. Principles of in-time analysis.

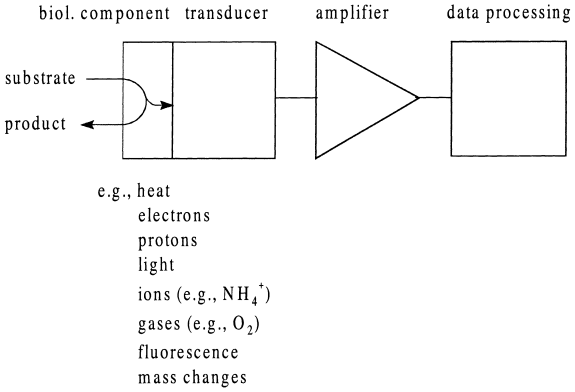

Fig. 4. Principles of biosensors.

delay between sampling and analysis when the data are needed to control the overall process. This fact is regarded by using the term in-time analysis.

3. Bioprocess monitoring

The monitoring of the physical environment of the cells is well developed and a huge variety of analytical tools is available to monitor process variables such as temperature, pressure, agitation, aeration and power input rates, liquid volume, foam level and broth turbidity [1,9,10]. In order to monitor the chemical environment and the biological state of the cells, the analysis of low as well as high molecular components in the medium or inside the cells (after cell disruption)

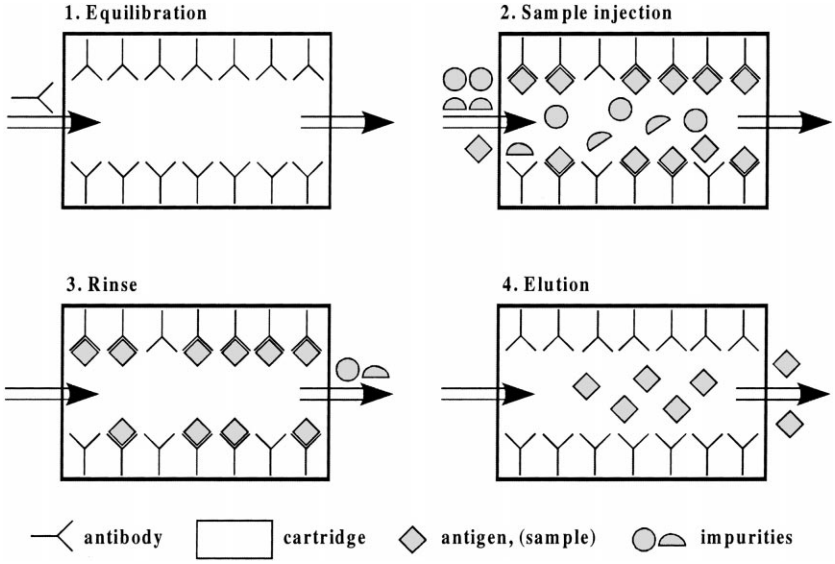

Fig. 5. Principles of an automated heterogeneous immunoassay.

is necessary. In general, electrochemical, optical sensors, biosensors or chromatographic procedures (e.g. HPLC, GC) are used to get information about the sample composition. Especially biosensors are extremely interesting for a sensitive and selective monitoring of single components even in complex bioprocess media. Excellent reviews are available which describe the function in general and different samples of application of these sensor classes [1,6,11–16].

Biosensors combine a biological sensing element (e.g. enzymes, cells, antibodies of receptors) and the appropriate transducer unit (electrochemical, optical). The biological sensing element reacts with the analyte. This recognition reaction (e.g. chemical conversion, mass change) is recognized via the transducer, amplified and correlated via calibration functions with the analyte concentration (see Fig. 4). Biosensors offer the possibility to analyze key components in a bioprocess to provide profound data for an efficient bioprocess control. Enzymatic biosensors are normally used to monitor low molecular weight components such as glucose, amino acids or antibiotics while immunosensors are used to monitor high molecular weight components. Different immunosensors are described for a quasi on-line monitoring of single proteins produced in a bioprocess by optical or electrochemical methods [17–24].

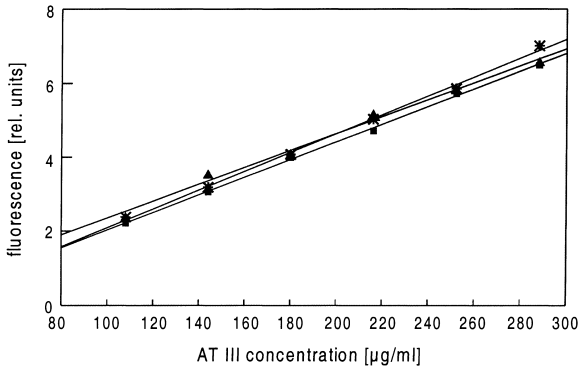

Fig. 6. Efficiency of the washing step of the heterogeneous immunoassay (■ without BSA addition; ▲ 1 mg/ml BSA addition; *10 mg/ml BSA addition).

4. Immunosensors

A variable fast and cheap automated immunoanalysis system was developed, based on the principles of flow-injection analysis and heterogeneous immunoassaying. The system was developed for the monitoring of high protein contents in the range of 1–1000 mg/l without dilution or enrichment steps. The principles of the assay systems are shown in Fig. 5. Antibodies are bound on polymer beads (100–250 μm diameter, VA-Epoxy Biosynth, Riedel de Haen, Seelze, Ger-

many). The immobilized antibodies are placed in a flow-through cartridge (1 ml volume Mobitec, Göttingen, Germany). The buffer is continuously pumped through the system. An aliquot of the cell-free sample from the bioreactor is injected into the carrier buffer flow, which transports the sample and the target protein present in it to the cartridge. The target protein will bind to the immobilized antibody, thus it is trapped inside the column up to a certain concentration. This critical concentration can be reached by varying the injected volume. The higher the protein concentration, the less is the volume to be injected. Thus, no dilution or enrichment steps are necessary. While the target protein binds to the immobilized antibodies all other byproducts are washed out by the buffer stream. After this washing step the antigen–antibody complex can be broken for example by a pH step in the carrier buffer system [22–24]. The proteins bound will be eluted, and flow through a spectrofluorometer placed at the outlet of the cartridge. The fluorometric signal can be correlated to the protein concentration by a calibration procedure. A short equilibration step is necessary before the next sample can be injected. In total, the analysis time is in the range of 3–4 min, that means 15 samples can be analyzed within 1 h. Fig. 6 shows the efficiency of the washing step. In order to check the efficiency different amounts of bovine serum albumine (BSA) were injected together with an antithrombine III (ATIII) containing sample [22–25]. However, the calibration graphs were nearly the same, even when extremely high concentrations of BSA were added. These results show that byproducts are washed out prior to the target protein analysis.

This heterogeneous assay offers tremendous advantages in comparison to conventional ELISA assays, since data are available within minutes in comparison to the long and labor intensive ELISA assays. Fig. 7 shows the comparison of the heterogeneous analysis to a conventional ELISA assay. These samples from a monoclonal antibody production process are given. The standard deviation of the heterogeneous assay was below 5% for all analytes tested up to now (e.g., AT III, rt-PA) [22–26]. The lifetime of these antibody cartridges is extremely high. Fig. 8 shows long-term stability tests over 600 injections. Here defined rt-PA concentrations were injected over a period of 48 h at room temperature, each 30th injection was used for recalibration of the overall system. Standard deviation

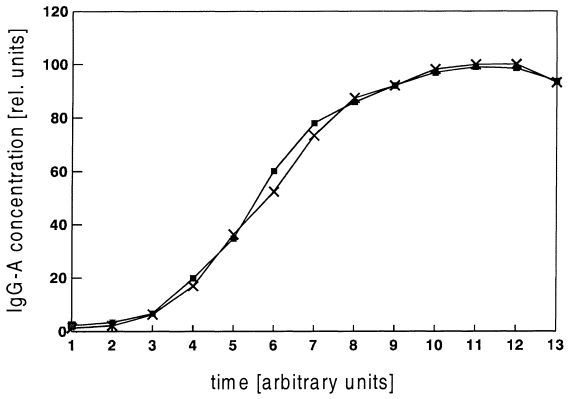

Fig. 7. Comparison between ELISA and heterogeneous assay data during the production of a monoclonal antibody. (■ ELISA; * heterogeneous immunoassay).

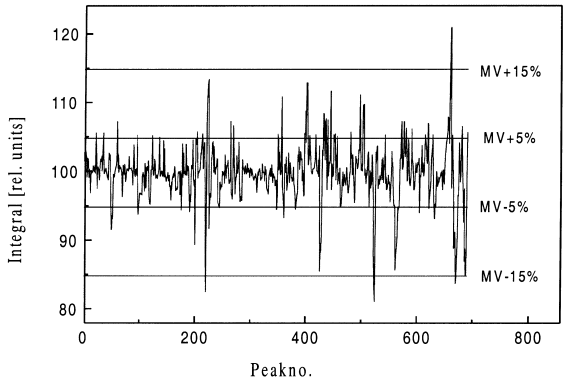

Fig. 8. Long term stability of the heterogeneous immunoassay (each 30th assay was used for recalibration, standard deviation: 4.1%).

in these experiments was below 5%, indicating that the whole heterogeneous immunoanalysis systems robust over a long period of time. It becomes obvious from these experiments that even complicated immuno analysis systems can be adjusted to the needs of a bioprocess, which means for mammalian cell cultivation processes a short analysis time, long lifetime, optimal concentration range in the area of 1–1000 mg/l, easiness of automation and reliability.

5. Optical sensors

During the last 15 years optical sensors have become more and more important for biotechnological

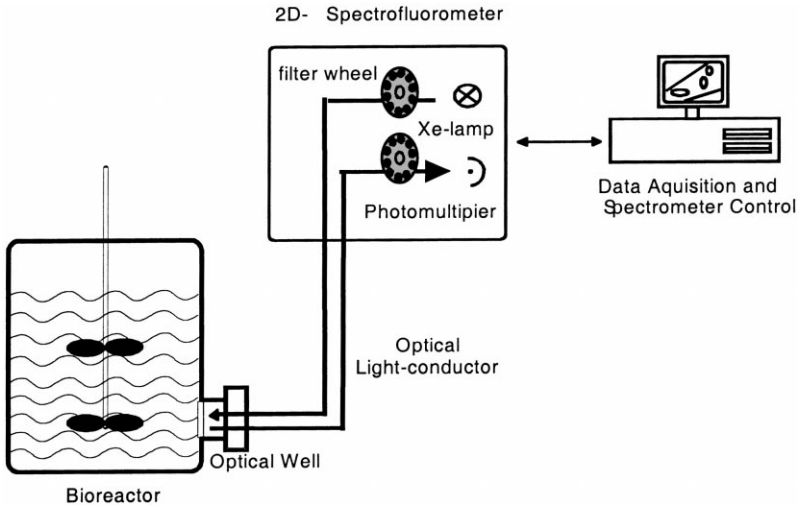

Fig. 9. Measuring setup.

applications. Optical detector systems can be interfaced via glass fiber systems to the bioreactor. All kind of spectroscopic analysis become possible by using this technique [27–29]. Transmittance as well as reflectance measurements are possible. A defined slit in the sensing fibers is used for transmission measurements, spectroscopic application and UV, IR region as well as for turbidity measurements are reported in the literature [27]. The monitoring of the reflected or the backward fluorescence light is used for turbidity as well as fluorescence measurements. The optical fibers are interfaced directly with the bioreactor, featuring a non-invasive in situ monitoring. Since optical assays do not have any time delay, real time data are available, using this kind of optical sensors [27]. In addition, optical chemosensors are described for application in biotechnology. Here a selective chemistry area is fixed on the fiber tip, forming a sensing element. Within the sensing element a special fluorophor is immobilized, which reversibly binds to the analyte. This binding detection reaction can be monitored spectroscopically by transmission or luminescent monitoring. Selective fluorescent sensors are described in the literature for oxygen and pH monitoring [27]. These sensors have tremendous advantages compared to conventional amperometric electrodes (e.g. miniaturization, high sensitivity, even under extreme conditions, no electrical contact with the sample).

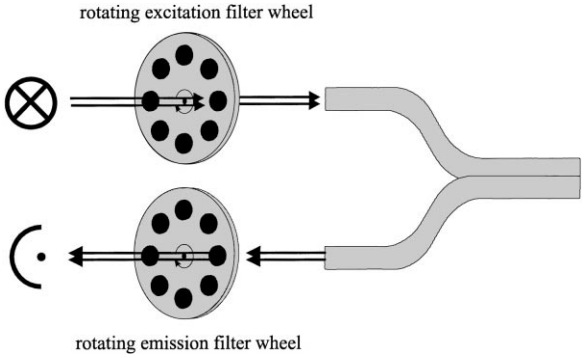

Fig. 10. Measuring principle of the BioView (only 8 filters are shown, the real instrument uses up to 16 filters).

6. 2-D-fluorescence monitoring

Fluorescence sensors are fiber optical measuring instruments which have been investigated over the last 15 years for different applications in biotechnology, such as biomass concentration determination, bioreactor characterization and metabolic studies (e.g., aerobic/anaerobic transition) and particularly bioprocess monitoring [28–32]. Those sensors monitored the metabolite NAD(P)H whose concentration gives information about the metabolic state of cells. In situ fluorimetry for the purpose of monitoring intracellular NAD(P)H levels was first reported by Duysens

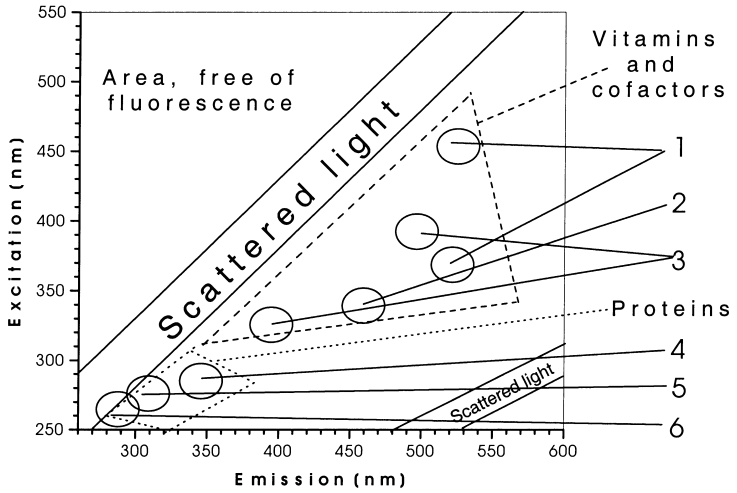

Fig. 11. Biogenic fluorophores in a 2-D-fluorescence spectrum (1: riboflavin, FAD, FMN; 2: NAD(P)H; 3: pyridoxine, pyridoxamine, pyridoxal-5′-phosphate; 4: tryptophan; 5: tyrosine; 6: phenylalanine), data taken from Schulmann [46].

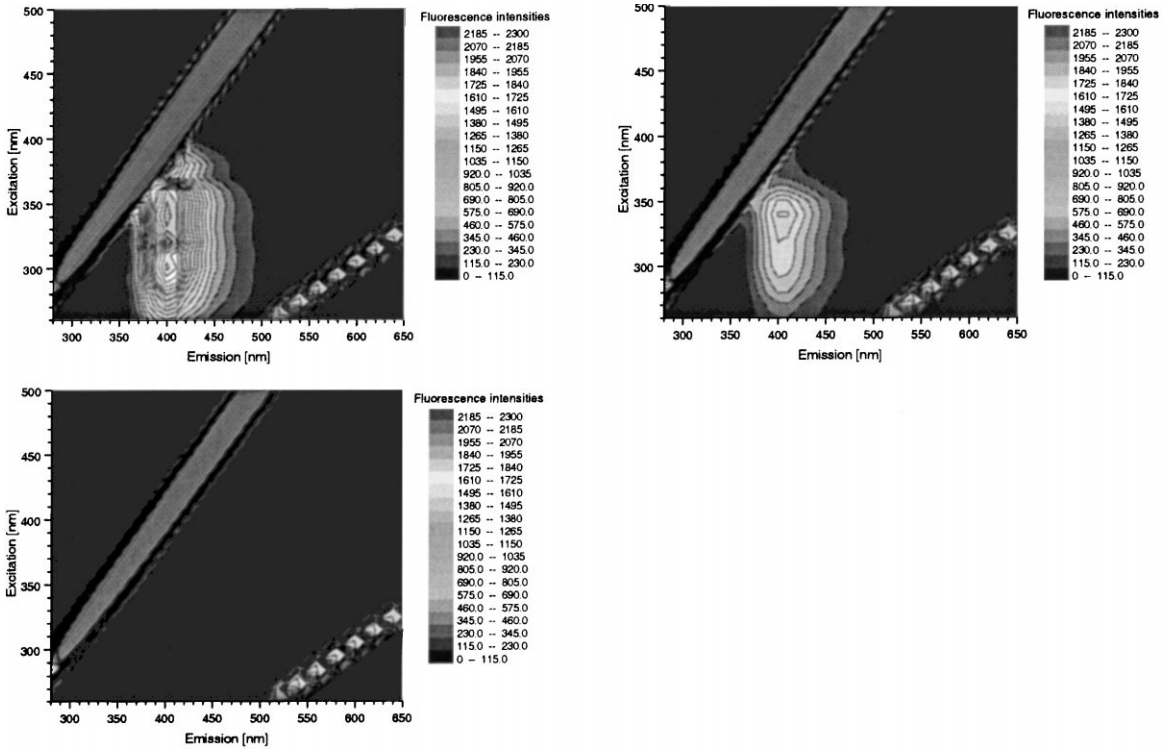

Fig. 12. Fluorescence spectra of *Sphingomonas yanoikuyae*(at 0 h (a), 12 h (b) and 24 h (c) of cultivation time).

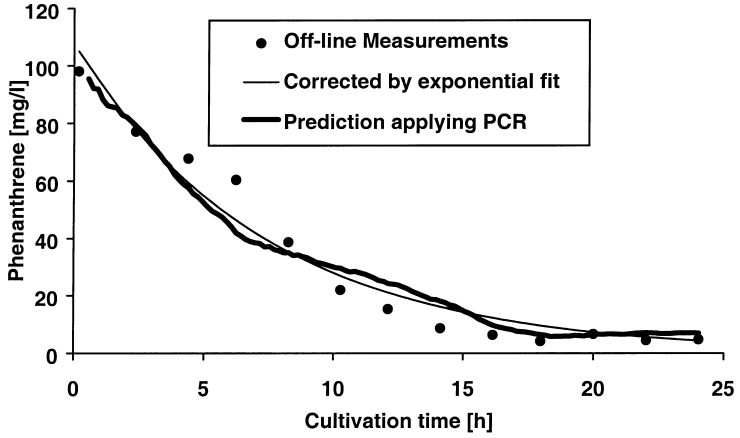

Fig. 13. Off-line measurements, the results of an exponential fit (thin line) as well as the prediction using principal component regression (thick line) of phenanthrene.

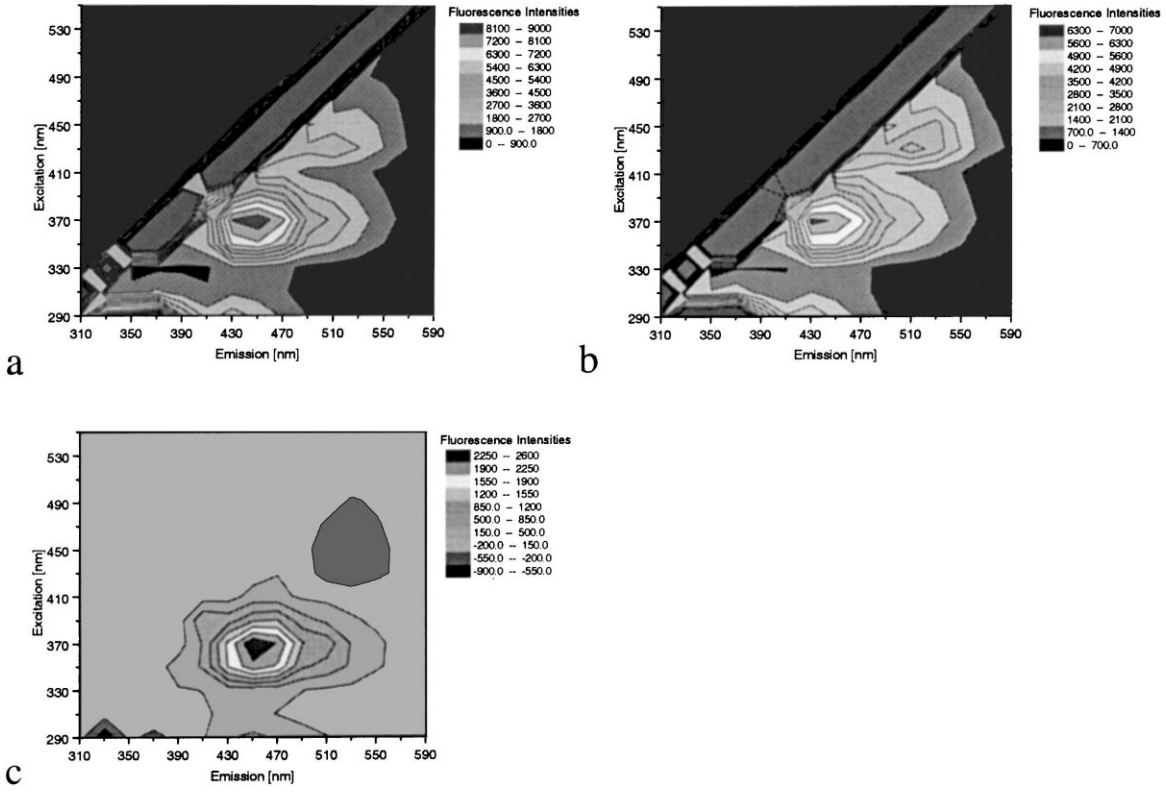

Fig. 14. Spectrum of *S. cerevisiae*: aerobic condition (a), anaerobic condition (b) and subtraction spectrum (anaerobic–aerobic condition) (c).

and Amesz in 1957 [33]. Irradiation of the bioreactor with UV light at 340–360 nm shows fluorescence of cell internal NAD(P)H at 450 nm. Harrison and Chance [34] were the first to use a fluorescence sensor for on-line monitoring of a cultivation in a glass bioreactor. The light source and phototube holder were applied at an angle of 60°. With this setup, measurements could only be done at low biomass con-

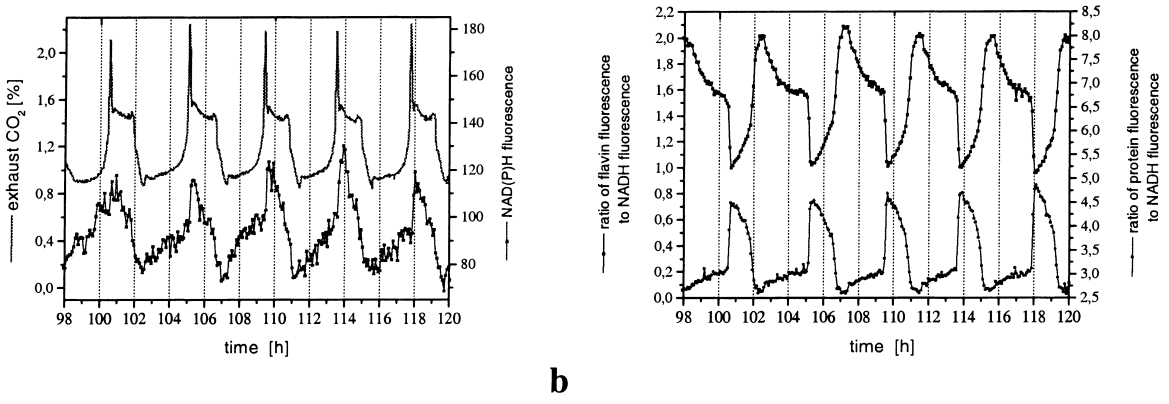

Fig. 15. Course of exhaust CO_2 and NAD(P)H fluorescence intensity (Ex370/Em450 nm) (a), and ratio of culture fluorescence intensities (b) during synchronized growth of *S. cerevisiae*.

centrations, as excitation light must penetrate a few centimeters into the cultivation broth. Changes from aerobic to anaerobic conditions of *Klebsiella aerogenes* could be observed. Zabriskie and Humphrey [35] used the setup for biomass determination of several different microorganisms. They measured culture fluorescence on-line at cultivations of *Saccharomyces cerevisiae*, one *Streptomyces*, and one *Thermoactinomyces* species. In the following years other applications for this technique were published by several research groups [32,36–40]. On the basis of these experiences, miniaturized fluorimetric probes were built [28–30,41] mainly for the monitoring of the NAD(P)H dependent fluorescence. Only few systems were described where different wavelenghts could be monitored [30,42,43]. Even when these techniques offer the possibility to non-invasively monitor the metabolic state of the cultivated microorganisms, the signal interpretation was often difficult. Fluorescence peaks are very broad in general, and there is a problem of overlapping, which is impossible to detect in a limited measuring range. The peak maximum shifts and other molecules might quench the fluorescence. Interactions of other fluorophores and changes of biological and physical parameters (like bubbles, pH, or dissolved oxygen) can also influence the signal.

These difficulties were overcome by the so called 2-D-fluorescence monitoring. Here all fluorophores present in the medium can be monitored simultaneously, giving information about the chemical environment as well as about the metabolic state of the cells. On the basis of this technique, a non-invasive sensor was developed which gains simultaneous information about the bioprocess. A 2-D-spectrofluorometer was developed (the so called BioView sensor) using independent filter wheels for excitation and emission up to 16 different filters each (Figs. 9 and 10) which can be designed individually. Usually measurements in steps of 20 nm are performed (excitation range: 270–550 nm, emission range: 310–590 nm), which was found to be most suitable without any loss of information. At the beginning of the monitoring of a complete 2-D-fluorescence spectrum, the excitation filter wheel is set to the first filter. The excitation light is guided via the fiber optic into the bioreactor. The backward fluorescent light is monitored via the emission filter which switches from filter to filter for a whole rotation cycle. Afterward, the excitation filter wheel switches to the next filter and the emission spectrum of the fluorescence produced by this defined excitation wavelength is monitored by one complete cycle of the emission filter wheel. This procedure is repeated until the excitation filter wheel has performed one complete cycle. Afterward a new spectrum can be measured. The number of measurements for each filter position can be individually chosen. The BioView sensor includes a software package, for sensor control and data analysis, which is able to predict the trend of the further course of the cultivation and is used to give information about times of interest (like harvesting time, feeding time, etc). A complete spectrum can be collected within 1 min [45]. In addition it is possible

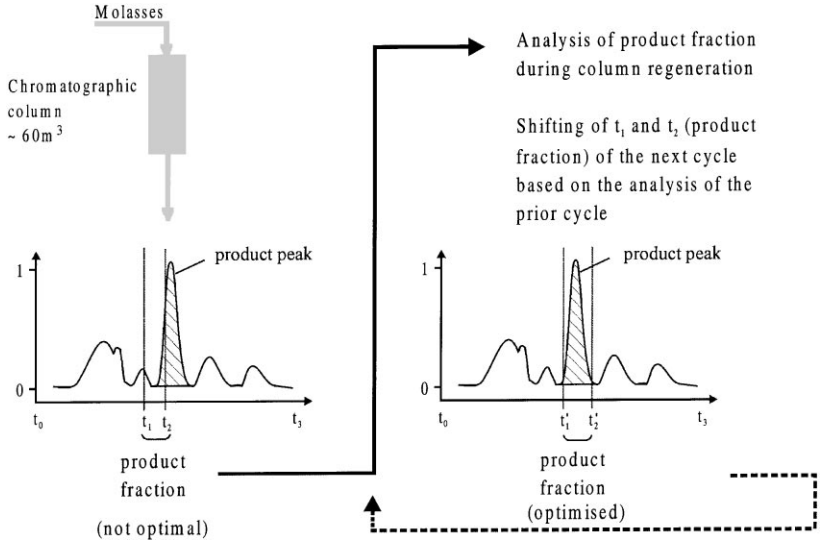

Fig. 16. Set-up of in time analysis during chromatographic processes.

to monitor single-wavelength pairs continuously. To avoid noise, smoothing can be performed by adjacent averaging. A high measuring frequency enables this procedure. If one spectrum is performed every 10 min, there are more than 150 000 measuring points (around 1.5 MB) in 24 h. Chemometric models (like the stepwise multiple regression procedure [44], principle component analysis (PCA), and neuronal networks) are used to filter information out of this mass of data and combine different regions in the matrix.

The spectral ranges of important biogenic fluorophores which might be present in the cell or in the chemical environment and which can be detected simultaneously by 2-D-fluorescence spectroscopy are shown in Fig. 11. Almost all biological processes depend on proteins, which fluoresce because of fluorescent amino acids such as tryptophan, tyrosine and phenylalanine. In another region of the 2-D-fluorescence spectrum (λ_{Ex} = 310–480 nm, λ_{Em} = 350–550 nm), vitamins (e.g., pyridoxine (vitamin B6), riboflavin (vitamin B2)) and coenzymes (e.g., NADH, NADPH, FMN, FAD) can be found.

The sensor was applied for the cultivation monitoring of *Claviceps purpurea*, *Escherichia coli*, *Saccharomyces cerevisiae* and *Sphingomonas yanoikuyae*. Cell growth and the metabolism of the cells (changes from aerobic to anaerobic conditions and uncoupling of the oxidative phosphorylation) could be detected.

The spectra derived during the cultivation of *S. yanoikuyae* are presented in Fig. 12. This organism is used for bioremedation since it effectively degrades phenanthrene. The decrease in the phenanthrene concentration becomes obvious over the time for the contour plots. Since more data about the concentration of other fluorophores are available, the chemometric data treatment allows to derive other important process information [47].

An example of the information extracted out of the 2-D-fluorescence spectra can be seen in Fig. 13. Here the results of principle component regression performed by using external validation (13 spectra for calibration model calculation, 120 spectra for prediction) is presented for the prediction of the phenanthrene concentration during its degradation. The off-line measurements of phenanthrene concentration seems to decrease roughly with a first-order kinetic, possibly due to the fact, that the mass transfer from the solid phase of the phenanthrene to the liquid phase is the limiting step in its degradation. Therefore, a first-order kinetic model has been applied to reduce the influence of the measurement errors. The off-line measurement values, the corresponding fit model values as well as the prediction of principle component regression values can be seen in Fig. 13. The obtained mean error of prediction is 2.2 mg/l, i.e. 2.1% (with respect to the maximum value). This demonstrates,

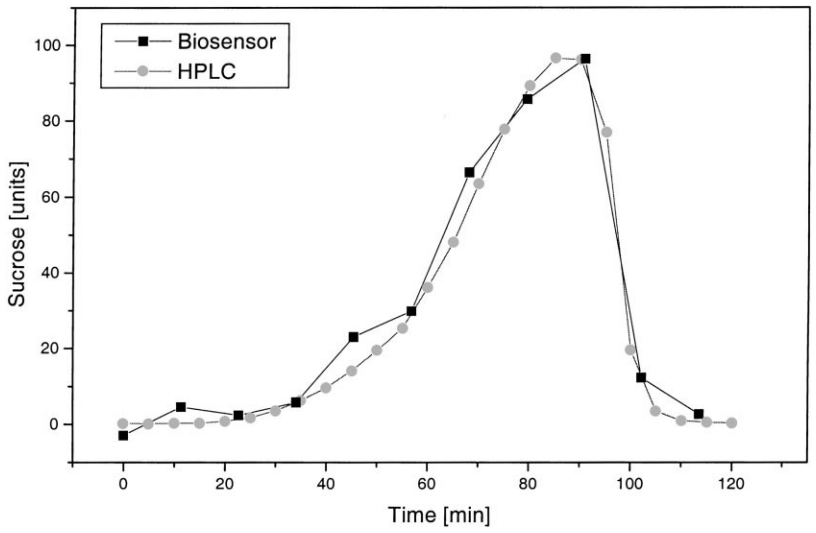

Fig. 17. In-time analysis of sucrose during chromatographic separation of sugar beet molasses.

that the exponential decay of the phenanthrene concentration is hidden in the spectra and very useful and precise information can be obtained out of the spectra.

2-D-fluorescence spectroscopy provides a direct insight into the cell during yeast chemostat cultivation and is sensitive enough to detect even changes in the metabolic flux of the cells. During aerobic conditions the reduced coenzymes (NADH, $FADH_2$) transfer electrons to O_2 during oxidative phosphorylation, where ATP is formed. Under anaerobic conditions (e.g. during aeration with nitrogen) this oxidative metabolic flux is interrupted. The concentration of the reduced forms (NADH, $FADH_2$) increases, whereas the oxidized forms (NAD^+, FAD/FMN) decrease. The spectra under aerobic and under anaerobic conditions seem to be similar. However, when the aerobic spectrum is subtracted from the anaerobic one, differences become obvious. A fluorescence increase can be observed in the NAD(P)H fluorescence region (Ex370/Em450 nm), since NADH accumulates, while a fluorescence decrease is obvious in the FMN/FAD region (Ex450/Em530 nm), since these metabolites do not fluoresce in their reduced form. These results are presented in Fig. 14 indicating the potential of this method to monitor even the metabolic state of the cells without disturbing the bioprocess.

The 2-D-fluorescence spectroscopy was also applied for the monitoring of oscillating yeasts as shown in Fig. 15. Here the overall dynamic behaviour of the synchronized cells becomes obvious. These studies are currently under investigation. The ratio of the flavin culture fluorescence (Ex450/Em530 nm) to the NADH fluorescence (Ex370/Em450 nm) shows the relation of the reduced coenzyme (NADH, $FADH_2$) to the oxidized coenzyme (NAD^+, FAD/FMN) in the oxidative phosphorylation during oscillatory behavior. 2-D-fluorescence spectroscopy allows even the detection of several parts of devision phase during one cell-cycle. The rate of NADH to protein fluorescence (Ex290/Em350 nm) increases rapidly at the beginning of DNA-synthesis (S-phase) and decreases rapidly at the end of S-phase. In the growth- (G_1) and division-phase (G_2,M) of the cell-cycle this rate is nearly constant.

7. Application of bioanalytics for industrial process optimization

Sugar beet molasses is a natural resource for various products used in daily life, ranging from sucrose to amino acids for pharmaceutical industry. The separation of molasses into these high value components is performed at the AMINO GmbH (Frellstedt, Ger-

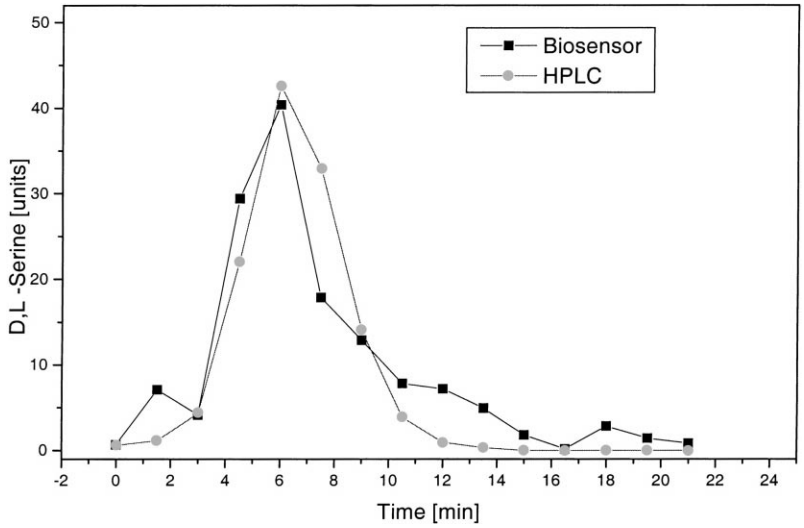

Fig. 18. In-time analysis of serine during chromatographic separation of sugar beet molasses.

Table 1
Process optimization of molasses desugarisation using FIA biosensor systems

Ecologic and economic profit	60% higher product concentration	25% higher product yield	35% less waste water
Reduction of...	200–250 t steam 30% specific energy for production process	3500 m^3 water of elution steps 110 t HCl 80 t NaOH 80 t NH_3 8 t charcoal	3500 m^3 waste water 180 t COD

many) in large scale (up to six 60 m^3, 60 000 t molasses per year) by ion exchange/exclusion chromatography. Typical products are different saccharides, nucleic acids and amino acids. The on-line control of the chromatographic process is based up to now on the monitoring of physical variables like density, conductivity, pH and refractive index. For example the sucrose concentration is estimated on line by polarimetry: the change in the rotation of light is correlated to the amount of sucrose in the sample. In the presence of other compounds interfering with light rotation (e.g. glucose and other sugars, lactic acid, ethanol), the measurement will not correspond to the actual sucrose concentration in the effluent stream. No method is available for the on-line analysis of amino acids like serine so far. The behaviour of the chromatographic columns can change from cycle to cycle (cycle time: 2 h), especially after complete regeneration or changes in the composition of molasses. An exact fraction cut of the product fraction is hard to perform, thus it might be necessary to rechromatography the whole fraction.

Thus, a flow injection biosensor system was set up for the in time analysis of serine and sucrose during the molasses desugarisation process. The set-up of this system is described in detail by Sosnitza et al. [48]. D-serine was analysed by converting it using the multi-enzyme system D-serine dehydratase/lactic dehydrogenase. The NADH consumed during this reaction stochiometrically was determined spectrophotometrically. Sucrose was determined with invertase/mutarotase/glucose oxidase and the oxygen consumed was monitored amperometrically. An analysis could be performed within 2–5 min by directly injecting samples from the chromatographic process into the flow injection analysis system. The standard deviation for the measurement of D-serine was 1.7%.

However, this analysis time is too long for real time control purposes, since the product fraction is eluted within about 5–10 min. For this reason, the biosensor system is used for 'in time' analysis: The product fraction is collected in 1 min intervals and analysed one after the other during two chromatographic cycles (cycle time 2 h). Based on the data obtained, the profile of the next product fraction is determined in advance. Thus the best fraction interval t_1 and t_2 (Fig. 16) can be obtained and adjusted to the actual state of the column. The whole process of sampling, analysis and adjusting t_1 and t_2 takes part during two chromatographic cycles and is finished in time, before the next product fraction is collected. The whole working principle of this system is shown in Fig. 16.

Both sensors are stable at least for 1 week under process conditions. Typically in-time analysis cycles for sucrose and serine are shown in Figs. 17 and 18. The knowledge of these two process variables leads to a better control of the molasses desugarization process. The time for collecting the product fractions can be determined more exactly. Thus, tremendous ecological and economical advantages were obtained (see Table 1) by using the intelligent, in-time biosensor system for supervising the downstream processing.

8. Conclusions

Bioanalytics offers interesting features for better process monitoring and understanding. Based on the detailed process data obtained, better process modeling and control can be achieved, resulting in more efficient processes with regard to product quality, product quantity and overall ecological and economical facts. Within the very near future more applications of intelligent bioanalytics will be given, showing the advantages of this sophisticated approach to process monitoring. Researchers and industrial users must cooperate to develop a bunch of modular system units which can be brought together to meet the special needs of each individual bioprocess.

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