Biosensor for on-line monitoring of penicillin during its production by fermentation

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Abstract:

An enzyme sensor has been developed for the on-line monitoring of penicillin V during its production by fermentation. The enzyme is crosslinked as a very fine film to the sensitive end of a pH transducer, using the spraying method. The biosensor is incorporated in a flow injection analysis (FIA) system within a home-made stirred flow detection cell. Penicillin-V in fermentation broth is detected during the whole fermentation process and the results are compared with the HPLC method. On-line measurements are achieved through the automation of the FIA system.

Key words: Flow injection analysis, enzyme electrode, penicillinase electrode, penicillin sensor, automation, fermentation monitoring.

INTRODUCTION

In the field of chemical analysis, biosensors have undergone rapid development over the last few years. This is due to the combination of new bioreceptors with the ever-growing number of transducers [1]. The characteristics of these biosensors have been improved, and their increased reliability has yielded new applications. Recently, a new technique of enzyme immobilization has been developed to obtain biosensors for the determination of enzyme substrates [2]. It is based on the enzyme adsorption followed by a crosslinking procedure. Therefore, a penicillin biosensor can be obtained and associated with a flow injection analysis (FIA) system for the on-line monitoring of penicillin during its production by fermentation [3-4]. This real-time monitoring of bioprocess would lead to optimization of the procedure, the yield of which could then be increased and the material cost decreased.

EXPERIMENTAL

Enzyme electrode

The determination of penicillin is possible by immobilising penicillinase onto glass electrodes. The principle of operation of the sensor is based on the changes in the H^+ concentration resulting from the enzymatic hydrolysis of penicillin by penicillinase .

Several methods for enzyme immobilization can be found in literature. In our laboratory, we have developed a new enzyme immobilization technique making possible response time of the biosensor much inferior to any of the response times so far reported for a penicillin sensor: the combined pH electrode to be coated with the enzyme was left overnight in sodium phosphate buffer at room temperature (used electrodes were dipped in 1M NaOH and 1M HCl, half an hour, each 3-4 times, alternatively, prior to its standing overnight in the buffer).

The electrode was then wiped dry with lens paper before being immersed for 15 minutes in an enzyme solution, containing 4 $mg.ml^{-1}$ of penicillinase. After drying the electrode for 20 min at 4 °C, it was mounted on a rotator horizontally, as shown in Figure 1.

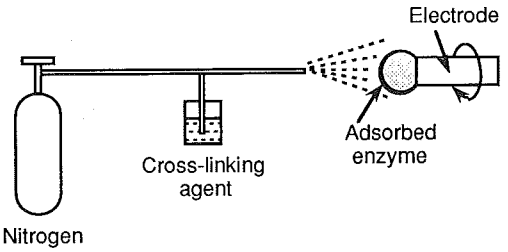

Fig 1. Deposition of thin enzymatic membranes in the construction of glass enzyme electrodes.

A solution containing 2.5 % glutaraldehyde in distilled water was then sprayed over the sensitive end of the electrode using an air-brush, under a pressure of 1.5 bar, nitrogen gas, at room temperature, keeping the electrode in rotation at 50 rpm. The diaphragm of the reference electrodes was covered by plastic clips to prevent a contact with the enzyme and glutaraldehyde solution during the immobilization period.

The reticulation between the enzyme and the glutaraldehyde over the electrode was allowed to continue for 15 min at 4 °C, before rinsing it in the buffer at room temperature for 5 minutes. The electrode was stored in the same buffer at 4 °C, when not in use.

Detection cell

The configuration is shown in Figure 2. Penicillinase was immobilized over a combined pH-glass electrode: type LoT 403-M8-S7, Ingold, Paris. A home-made stirred flow cell is used as detection chamber. PVC and PTFE were used to construct the cell; small stainless steel tubes were used as fluid inlets (in order to connect the tubes to the cell). The potentiometric measurements were obtained using a Radiometer pHM-64 research pH meter connected to a Sefram-Recorder (Sefram-Servotrace, Paris) and to the computer. The detection cell was agitated by a stirrer (Microlab, Aarhus, Denmark) at a moderate speed.

Sample and buffer solutions were pumped by two 2-channel peristaltic pumps (Ismatec SA, Zürich, Switzerland). using tygon tubes 0.51, 1.42 and 1.52 mm inner diameter (Bioblock, Illkirch, France).

Automation procedure

Automatic sample injection is carried out by use of a timing control pneumatic actuator connected with a 4-way teflon injection valve (both Rheodyne, Cotati, U.S.A.). The actuator permits automatic operation of the valve.

The analog millivolt signal arising from the pH-meter is converted to digital equivalent by a 6B11 module mounted on a 6BP04-2 backplane. The digital I/O backplane is a 6B50-1 module which provides 24 digital I/O channels. These backplanes are from Analog Devices, Norwood/U.S.A. The I/O channels are connected to solid-state relays plugged in a EGS08000 backplane from Celduc, Sorbiers/France. These relays control the injection valve and the pumps.

A MacIntosh computer is connected to the 6BPO4 backplane via the serial port (RS-232).

The software is written in Quick Basic, and it is structured, with subroutines for control of the injection valve and the pumps, data aquisition, calibration, and calculations.

Subroutines ensure recording of the baseline, injection of sample, recording of response signal and preparation of the next sample injection. The record signals are saved and by means of calculation and calibration subroutine, the mV-peak height is converted to the appropriate concentration of the substrate. Four calibration samples with different concentrations are used to trace the calibration curve.

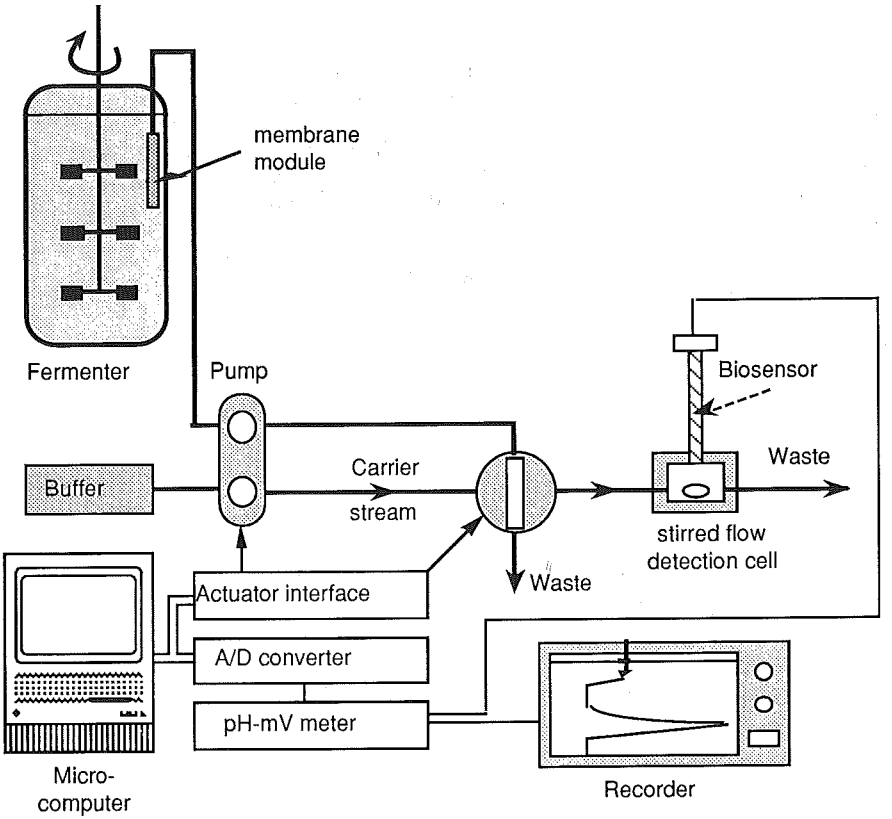

Fig. 2: Automated Biosensor-FIA system for penicillin monitoring

Flow injection system

The manifold is shown in Figure 2. The baseline was obtained by pumping a buffer solution through the detection cell (pH meter in millivolt mode). The sample flow rate was always 0.78 ml.min^{-1}. Penicillin standard solutions of 0.1 mM to 65 mM were prepared in the fermentation broth. The diluted samples were injected into the carrier by means of the injection valve which is placed close to the detection cells in order to minimise the delay between injection and detection. The dead volume chosen was 2 ml. The cells are thermostatted (25°C). For measurements, the potential difference between the peak height and the base line was automatically recorded by the computer and the recorder. The potential of the electrode always returned to its base line as soon as a fresh buffer solution come again to the contact of the electrode.

Penicillin in fermentation broth were measured after sample withdrawal from the bioreactor. The samples were filtered by an *in situ* membrane module with a 0.22 µm nominal pore diameter.

RESULTS AND DISCUSSION

The necessity to measure penicillin in fermentation broth is important. Penicillin fermentations typically run for 10 days and a high frequency of analysis is therefore not necessary. However, this determination is done within a large concentration range and in a complex media. During the fermentation process, there is a significant change in the medium composition, whereas the pH value of the broth remains approximately constant. To fulfil these requirements, injection volume, flow rate and buffer strengths were adjusted to have good reproducibility as well as acceptable sensitivity and response time.

The penicillin calibration curve, conducted before the beginning of the fermentation monitoring, is shown in Figure 3. It can be seen that there is a large linear measuring range: the maximum penicillin concentration at the end of the fermentation is about 60 mM. This fact presents an important advantage in view of simplicity of the fermentation monitoring because one single calibration curve covers the whole concentration range.

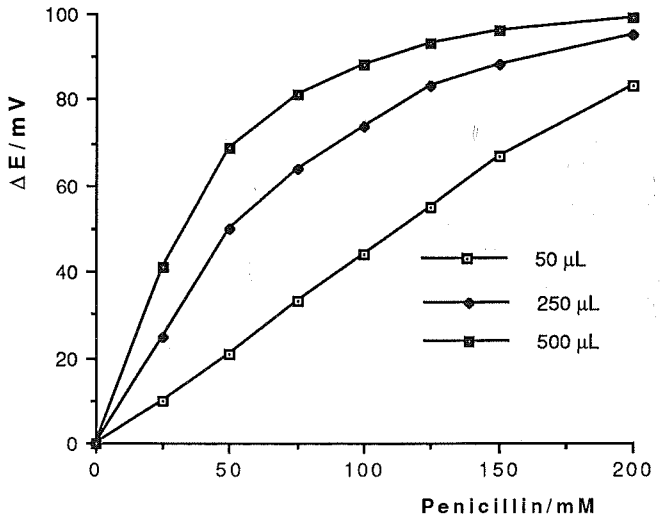

Fig. 3: Calibration curve for the penicillin sensor as a function of sample injection volumes: (50, 250, 500 µL)

The comparison of the results of the total penicillin V concentration versus the fermentation time, obtained by the reference method (HPLC) and by the biosensor-FIA system is shown in Figure 4 for the fermentation FBO-23. Penicillin-V together with a small concentration range of para-hydroxy-penicillin-V are produced during the fermentation. Both penicillin V possess a ß-lactam ring and are detected by the biosensor. The Figure shows good agreement between the two off-line measurements over a wide range of concentration with a better repeatability of the biosensor measurements. The operating conditions chosen for FIA allow measurements without any change in parameters during the whole fermentation process. The pH of the broth is regulated to about 6.5± 0.2. The Figure shows good agreement of the biosensor responses over a wide range of concentration with the HPLC measurements.

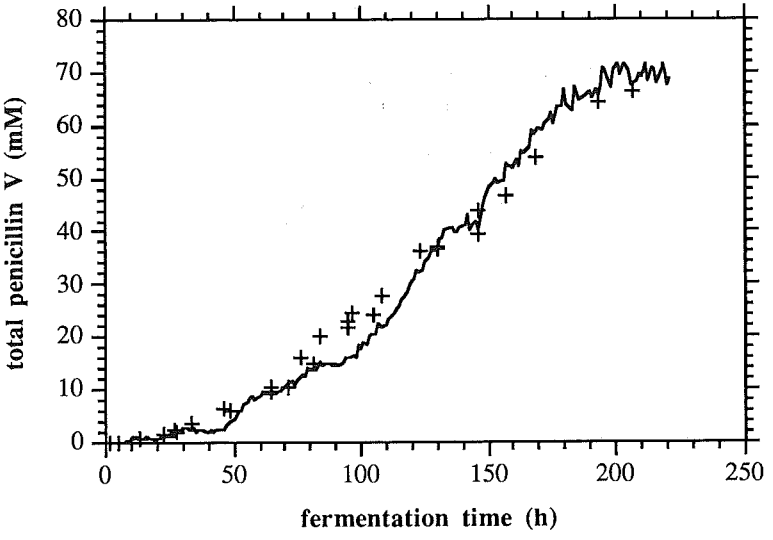

Fig. 4: Determination of penicillin V during the fermentation process with a biosensor (——) and HPLC (+) methods

Stability of the biosensors

The long term stability of the two electrodes was examined at room temperature. After construction, 2 penicillinase electrodes were used in the FIA system for about 8 hours per day. Between measurements, the sensors were stored in the detection cells, always at room-temperature. The penicillinase electrode shows a good stability (98% of initial response) during 33 days.

CONCLUSION

Flow injection analysis (FIA) combined with biosensors offer new applications in the field of automated analysis and process control. Biosensors with rapid response time ensure high sample throughput, improve the sensitivity and decrease the cost of analysis.

Using a new immobilisation technique, it is possible to obtain a fast responding enzyme sensor using an ordinary pH glass electrode. This type of sensor can provide substrate concentration after only a few seconds. Enzyme electrodes are directly incorporated in a home-made stirred flow detection cell. Automation of the biosensor-FIA system make possible on-line monitoring of penicillin-V during its production by fermentation. The various conditions required for penicillin determination during the fermentation process are reduced or completely eliminated by the sample dilution, which takes place in the detection cell. Investigations concerning the optimal operating conditions of the FIA system have also revealed so that the operating parameters can be maintained over the whole penicillin concentration range.

The present biosensor/FIA system is simple, reliable and inexpensive to determine penicillin in fermentation broth samples and it can also be used for the determination of other biological species [5].

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