On-line Monitoring of Glucose and Acetate during High-Cell density Fermentations of *Escherichia coli* with a Flow Injection Analysis System

Isabel Rocha and Eugénio C. Ferreira

Centro de Engenharia Biológica – IBQF, Universidade do Minho 4710 – 057 Braga PORTUGAL

Abstract

A system based on Flow Injection Analysis (FIA) for the on-line monitoring and data acquisition of acetate and glucose concentrations in the liquid phase during high cell density fed-batch fermentations of recombinant *E. coli* was developed.

A photometric detector is used for the measurement of acetate. It detects the decrease in the absorbance of phenol red, which is proportional to the amount acetate initially present in the sample stream. After method optimisation, it was possible to achieve linearity until 10 g/kg without needing a dilution step and with a sensibility of 0.05 g/kg. No significant interferences were detected when compared with other methods.

Commercially packed Glucose Oxidase is used in the amperometric measurement of glucose. The method is linear until 5 g/kg and it is possible to detect concentrations of less than 0.1 g/kg.

The FIA system is coupled with a computer that acquires and stores the results, which are finally accessed by the supervisor computer where a main program allows the development of control actions based on the concentration of the two compounds, together with other on-line information.

Several fed-batch fermentations with recombinant *E. coli* were conducted with success using the system described. The measurement ranges revealed adequate without needing a dilution step and the filtration system was stable even at high cell densities (more than 55 g/L of biomass).

I. Introduction

The success of a recombinant fermentation depends highly on fast and accurate measurements of the major state variables. Flow Injection Analysis (FIA) has become one of the most popular techniques for the on-line measurement of substrate and products in biotechnological processes due to its flexibility, feasibility, and short analysis time. However, in order to take the necessary control actions in real time, it is necessary to integrate the generated data in a complete data acquisition system.

A FIA based system for the on-line monitoring of glucose and acetate during the production of recombinant proteins in fed-batch with *E. coli* was optimised and integrated with data from exit gas analysis, digital control unit and weight readings.

II. Materials and Methods

The FIA system for the measurement of acetate is illustrated in fig. 1. It is based on the diffusion of that volatile compound through a gas-diffusion chamber into a stream containing an acid-base indicator. The subsequent decrease in the absorbance is detected with an incorporated photometer. Glucose is measured amperometrically by the presence of hydrogen peroxide produced from glucose by Glucose Oxidase (fig. 2).

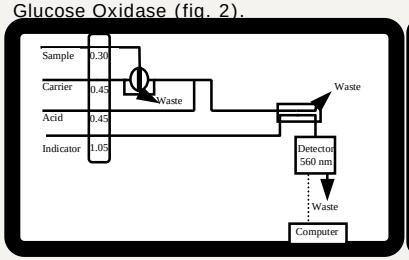


Fig. 1 FIA system for the measurement of acetate

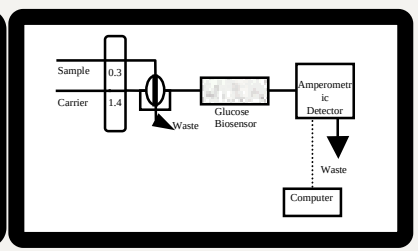


Fig. 2 Method used in the measurement of glucose with FIA

III. Results

Table 1 shows the results obtained during the optimisation of the method used for the analysis of acetate. Clearly, the buffer capacity of the indicator determines the linearity and, on the other hand, the detection limit of the measurement. In figures 5 and 6 it is shown a typical calibration for each one of the FIA methods used.

Table 1. Linearity and detection limits as a function of the buffer capacity of the indicator for the acetate determination with FIA

Phosphate Conc. (mM)	r^2 until 10 g/kg	Linearity Limit (g/kg)	Detection Limit (g/kg)
0.25	0.9716	2.5	0.08
0.375	0.9862	2.5	0.04
0.5	0.9841	5	0.06
0.75	0.9966	7.5	0.08
1	0.9986	10	0.1
1.25	0.9995	10	0.2

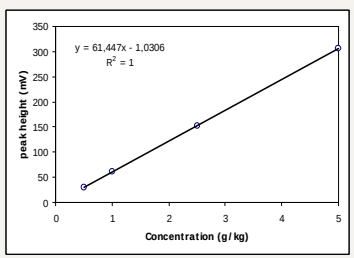


Fig. 5 Typical calibration obtained for the glucose measurement with FIA.

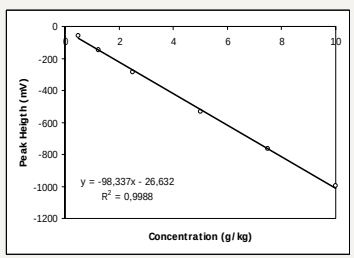


Fig. 6 Results obtained during the calibration of the acetate method.

Fig. 3 represents the experimental setup for the fed-batch cultivation of *E. coli*. Retrieval of liquid medium samples free of biomass and suspended particles is obtained by on-line filtration of the fermentation culture using an external unit, composed of a peristaltic pump and a tangential filtration device (A-SEP). The exhaust gas line is connected to the Mass Spectrometer for the measurements of the oxygen and carbon dioxide transfer rates. The Digital Control Unit (DCU) measures and controls environmental properties like pH, temperature, agitation speed and dissolved oxygen concentration. Two balances continuously measure the weight of the fermentation culture and the feeding tank. The resulting data obtained are directly acquired by the supervisor computer with several *ad-hoc* sub-programs developed in LabView environment. The FIA system is coupled with a proper computer equipped with appropriate software that continuously writes data in a file. These data are then accessed via network by the supervisor computer.

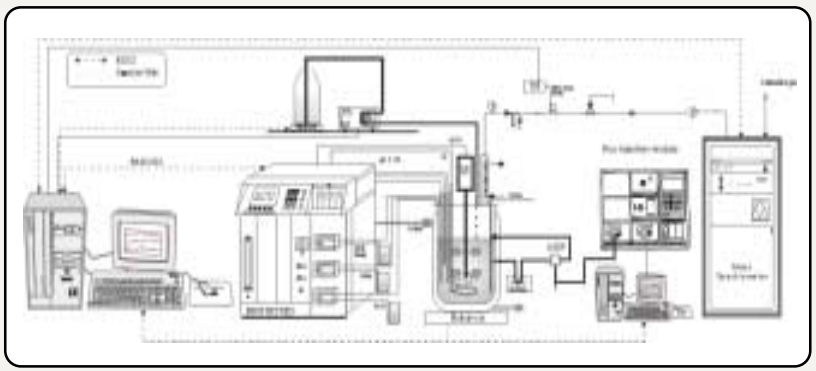


Fig. 3 Experimental setup for the fed-batch fermentation of *E. coli*

Fig 4 shows the front panel of the main LabView program, where all the sub-programs are integrated, allowing a complete monitoring of the fermentation process.

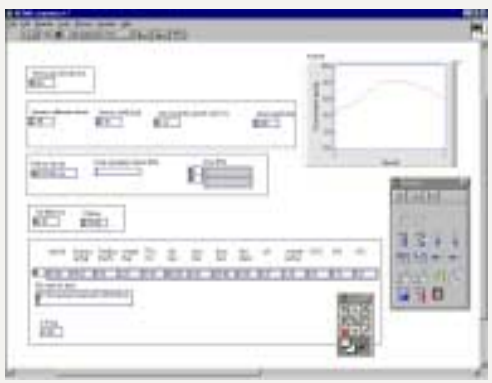
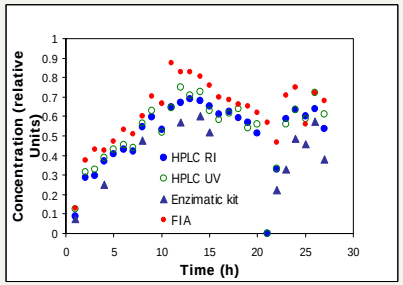


Fig. 4 Front panel of the main VI developed in LabView for the on-line data acquisition and control of the process.

In figures 7 and 8, the acetate method is evaluated both on-line and off-line by comparing the results obtained during a fed-batch fermentation of recombinant *E. coli* with other methods like UV-HPLC, RI-HPLC, and a enzymatic kit (Boehringer Manhein).



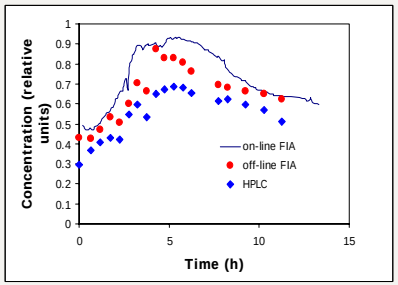


Fig. 7 Comparison between several methods for the measurement of acetate in the course of a fermentation

Fig. 8 Comparison between on and off line analysis of acetate concentration with FIA.

IV. Conclusions and Future Work

The method used for on-line acetate measurements with FIA revealed very versatile, being the buffer capacity of the indicator a key variable. Hence, it is possible to measure low concentrations of acetate with higher sensibility or higher concentrations (until 10 g/Kg) with less sensibility.

Measurements of acetate with FIA are also feasible, when compared with other methods. Namely, the difference found between HPLC (the most commonly used method) and FIA is similar to the one encountered for HPLC and the highly specific enzymatic kit from Boehringer Manhein.

There is a significant difference between on-line and off-line measurements with FIA, probably due to the relatively long dead time between sampling and analysis. That is one of the problems to be solved in the next future.

The system for data acquisition based on LabView programs revealed adequate for the on-line monitoring of fed-batch fermentations of recombinant *E. coli*.

Acknowledgements

This work was supported by the PROTEXPRESS project (Agência de Inovação).

The FCT provided financial support for I.Rocha through a doctoral research grant.

International Conference on Measurements, Analysis, and Control in Bioprocesses Technology – Current Status and Future Prospects

Cambridge, UK, 24-26 September 2000