Measurement of Acetate during the production of Recombinant Proteins with *E. coli*

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Abstract

A method based on Flow Injection Analysis (FIA) was adapted and optimized for the on-line monitoring of fermentations of recombinant *E. coli*.

The method is based on the pre-acidification of the sample with sulfuric acid followed by the diffusion of the acetate into a stream containing an acid-base indicator through a hydrophobic membrane. The decrease in the absorbance of the acid-base indicator at 560 nm is proportional to the concentration of acetate and is measured with a photometer for that wavelength.

The composition of the indicator was studied in order to achieve a compromise between stability and sensitivity of the method. Several solutions are proposed, depending on the concentration range of acetate to be measured. It was possible to achieve linearity until 10 g/Kg of acetate with a sensibility of less than 0.1 g/Kg.

The correlation between acetate measured with FIA and with other methods (HPLC and enzymatic kit from R-Biopharm) is also acceptable, the differences never exceeding 20%.

The method is very reproducible, being the averaged relative standard deviations around 2% for 20 replicates of the same sample. The method is also very fast, being the sampling rate of 30 h^{-1}.

I.Introduction

Acetate is known to be the major by-product during fermentation of recombinant *E. coli*. Among the consequences of that production, the more important are the decrease of the biomass yield for a given carbon source, the inhibition of the growth when acetate is present at high concentrations (typically 10 g/L) and the decrease of the production of recombinant proteins. For these reasons, its accurate and fast measurement is a very important issue when trying to achieve high productivities in this kind of processes

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 a phenol red solution. The subsequent decrease in the absorbance is detected with an incorporated photometer.

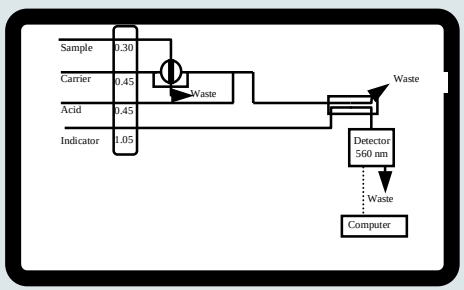


Fig. 1 FIA system for the measurement of acetate

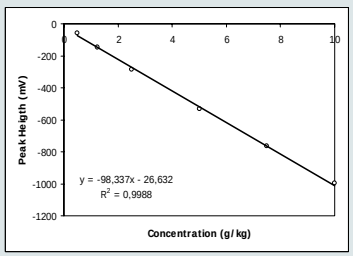


Fig. 3 Results obtained during the calibration of the acetate method.

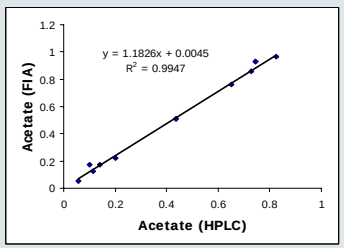


Fig. 4 Correlation between measurement of acetate with FIA and HPLC

In figures 5 and 6, 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 (R-Biopharm).

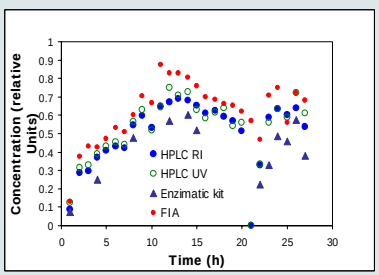


Fig. 5 Comparison between several methods for the measurement of acetate in the course of a fed-batch fermentation

During the fermentation, 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 fermenter coupled to the Digital Control Unit and the FIA system used in this process is shown in figure 2.



Fig. 2 Cultivation of *E. coli* with measurement of acetate 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 figure 3 it is shown a typical calibration for the FIA method used. The correlation between HPLC and FIA for a batch fermentation is shown in figure 4.

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

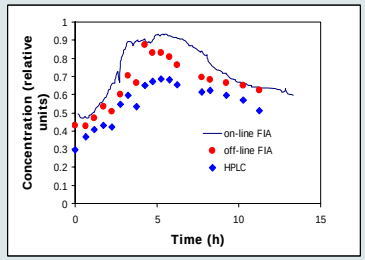


Fig. 6 Comparison between on and off line analysis of acetate concentration with FIA during a fed-batch.

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 R-Biopharm.

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.

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 (PRAXIS XXI/BD/16961/98).