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

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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.

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.

The measurement of acetate 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. After method optimization, it was possible to achieve linearity until 10 g/L without needing a dilution step and with a sensibility of 0.05 g/L. 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/L and it is possible to detect concentrations of less than 0.1 g/L.

The FIA system is coupled with a computer equipped with appropriate software that continuously writes data in a file. These data are then accessed via network by the supervisor computer running a programme developed in *Labview* environment. This software allows the development of control actions based on the concentration of the two compounds, together with on-line information from exit gas measurements using mass spectrometry, on-line weight readings from two balances (one for the fermenter and the other for the feeding substrate), and other state variables acquired from the digital control unit coupled to the fermenter.

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).