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

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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.

A method based on Flow Injection Analysis (FIA) was then 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/L of acetate with a sensibility of less than 0.1 g/L.

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}.