RAMAN SPECTROSCOPY FOR ON-LINE MONITORING OF AMINO ACID CONCENTRATIONS AND ANTIBODY N-GLYCOSYLATION IN HIGH CELL DENSITY PERFUSION PROCESS

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Process analytical technology (PAT) is very important for continuous bioprocesses. Raman spectroscopy has been reported for fed-batch processes however its usage for perfusion processes does not seem largely used. A reason could be that steady-state perfusion processes generate constant metabolite profiles while dynamical data are required to calibrate the partial least square (PLS) models used for Raman spectroscopy. The present study focused on integrating process development of high cell density perfusion culture and the calibration of PLS model for Raman spectroscopy. The process used CHO-K1 cells producing antibody in bioreactors (200 mL) using ATF2 as cell separation system and equipped with Raman probes (Kaiser+Endress). The process development explored the effects of cell density, perfusion rate and cell specific perfusion rate (CSPR) on the culture performance, while the glucose was delivered using a targeted feeding as previously reported [1]. Steady-states at different cell specific perfusion rates (CSPR) were carried out by varying the perfusion rate and the cell density, which stretched from 2 to 100 x 1E6 cells/mL. This study showed that the cell density and the perfusion rate did not impact the process performances, while their ratio, the CSPR, had a large influence on these performances.
The data obtained during this process development were used for the calibration of the PLS model for the different culture parameters, i.e. cell density, metabolites including the amino acids. It was showed that the model could efficiently predict these parameters for another culture run in cross-validation very accurately. It is probable that the training data set generated at different steady-states from different CSPRs provided dynamical features for the model calibration, which was valid over a wide range of metabolites and cell concentrations. The cell density, lactate, ammonium and amino acid concentrations were predicted at different CSPRs with overall low prediction errors less than 15% for most components in cross-validation. A supplementary observation was a high correlation between the amino acid profiles and the antibody N-glycosylation. This was exploited to create a new PLS model of the glycosylation using the amino acid concentrations in the culture as inputs, derived either from on-line prediction of their value based on the Raman probe or based on their off-line measurements. This model was successfully able to predict the glycosylation profile in cross-validation.

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