Qualitative monitoring of bio-processes by using near infrared spectroscopy

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Introduction

The near infrared (NIR) region of the electromagnetic spectrum lies between the visible and the infrared (IR) region, i.e. between 785 and 2500 nm. Absorption in the NIR region is much weaker than in the IR region. This enables analysis of samples that are strongly absorbing and highly light scattering such as fermentation broths. Analysis of samples can be performed without any pre-treatment such as filtration or centrifugation of the samples, i.e. the analysis can be conducted on-line. The use of optical fibres within the NIR region allows on-line connection of the flow cell or transmission probe.

The potential to monitor and control biotech processes has been limited to on-line instruments created for the chemical industry, measuring temperature, pH, dissolved oxygen (DOT), the redox potential etc. However, these parameters monitor the fermentation condition and do not yield any information about the cell status, unless combined with measurements of cell concentration. To further develop and optimise biotech processes, the need for on-line sensors that monitor cell numbers and cell status, nutrients, by-products and other aspects important for a certain process, are essential. Although there are systems available for on-line measurements of cell number, nutrients and by-products these instruments are fairly complicated and need a continuous sampling system. NIR spectroscopy, in combination with chemometrics, offers the potential to monitor processes on-line and is much less labour demanding than systems that need to be carried out off-line or that need continuous sampling systems at the same time as providing a direct answer from the process. This enables the appropriate action to be taken once a deviation or shift from the optimum path occurs. Another set back for sampling systems and off-line analysis is the representivity of the sample. This is especially true for samples that are saved for analysis at a later date. Also, the demands for keeping the process sterile is in favour of the on-line, non-invasive NIR spectroscopy. NIR spectra may be collected in the actual process or in a process stream. In process analytical chemistry (PAC) the use of chemometrics, combined with unspecific measurements methods such as NIR spectroscopy has significantly improved the way fermentations can be monitored on-line. NIR spectroscopy provides a non-invasive, on-line monitoring device able to characterise the process in both a quantitative and qualitative perspective.

The focus of NIR spectroscopy within biotech applications has been to quantify key parameters[1–12] such as cell number and medium composition. NIR spectroscopy enables quantification of several key parameters at the same time. However, the work with calibration and creating reliable models should not be underestimated and, preferably, routine analysis should be conducted to verify the monitoring capability of the developed calibrations. Within organic chemistry, the study of principal component plots has created a tool for process monitoring where the propagation of the reaction can be studied and

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a possible deviation can be detected.[13–15] Similar studies have been conducted on bioprocesses with unspecific sensors, such as the electronic nose.[16] The combination of unspecific measuring methods such as NIR spectroscopy with chemometrics creates the possibility to both monitor and, at a later stage, to control the process by parameters related to quality. In biotech processes such parameters may be related to the amount, stability and quality of the product.

Experimental

Instrumentation

The NIR spectrophotometer used was an InfraPrime from Bran+Luebbe. Experiments were evaluated using both a flow cell (Bran+Luebbe) and a transmission probe (Hellma Tauch 661). The experiments were conducted on-line and both the flow-cell and the transmission probe were connected to the spectrophotometer with a 5 m optical fibre, Figure 1. The flow-cell was positioned in an external circulation loop to the bio-reactor and the transmission probe was positioned through a fitting on the side of the bio-reactor. The flow cell was positioned at the entrance of the by-pass to avoid the cell culture changing at the point of collection of spectra. The flow-cell and transmission probe both used a 1 mm path length and the spectra 900–1700 nm were collected with a 2 nm interval.

Cell culture

The studied mammalian cell culture is used for the production of a recombinant protein at Pharmacia & Upjohn.

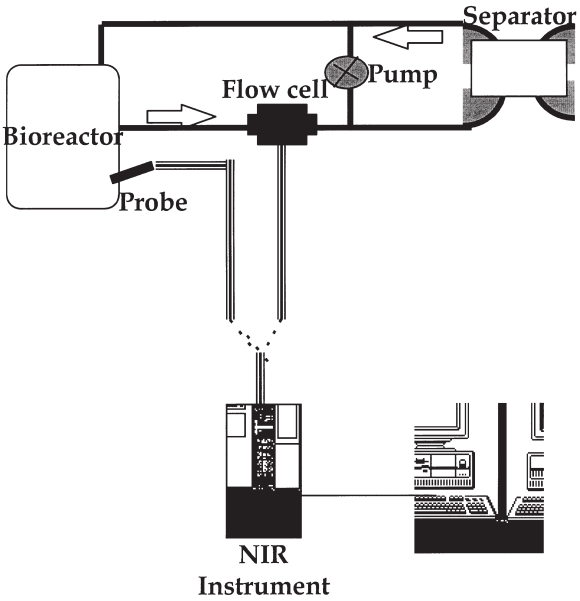

Figure 1. External loop with flow cell and the transmission probe.

Spectra collection

A total of 159 spectra were collected for the flow cell and a total of 100 spectra were collected for the transmission probe. Spectra with the flow cell and the transmission probe were not collected for the same batches. Some of the spectra were collected at the time of sample removal for off-line analysis. These spectra together with the results from the off-line analyses were used to create quantitative calibrations. The quantitative results are reported in the article "The use of NIR spectroscopy in monitoring and controlling bio-processes".[12]

Data treatment

Principal component analysis (PCA)[17] was conducted with the software, Simca-P (Umetri AB). The additional mathematical pre-treatments of absorbance data were; normalisation, smoothing, first derivative, second derivative and normalisation combined with the second derivative. Pre-treatments were calculated by using the Savitsky–Golay algorithm.[18]

Results and discussion

As the fermentation proceeds, cell number, cell debris, product formation and by-product formation increase and the appearance of the broth gradually changes from a clear solution to one that has a more opaque appearance and that is strongly light scattering. Due to this, the absorbance increases at all wavelengths during the course of the fermentation.

Principal component analysis (PCA)

Depending on the pre-treatment used, the chemometric tool, principal component analysis, made it possible to characterise different phenomena. Using the flow cell, the process changes (C1) and two different phases, the batch cultivation phase (P1) and the perfusion cultivation phase (P2) in the process were clearly visible (Figure 2). Additionally, a disturbance in the process was detected and the transfer back to normal conditions was monitored. Using the transmission probe the same process phases and changes could be detected. During the transmission probe experiment an infection in the cell culture could be detected prior to detection in any of the univariate off-line sensors; dissolved oxygen, temperature and pH.

Figure 2 is based on absorbance data and the change in the path for the two phases is apparent, together with the process change C1 and the disturbance.

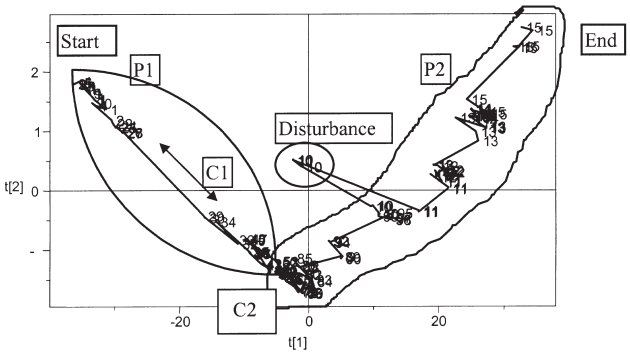

Figure 2. Scores plot for the two main principal components from absorbance data collected with the flow cell.

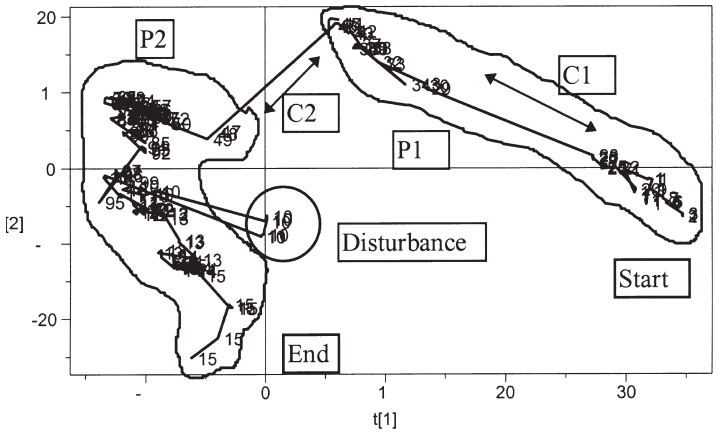

Figure 3. Scores plot for the two main principal componenets on first derivative pre-treated data collected with the flow cell.

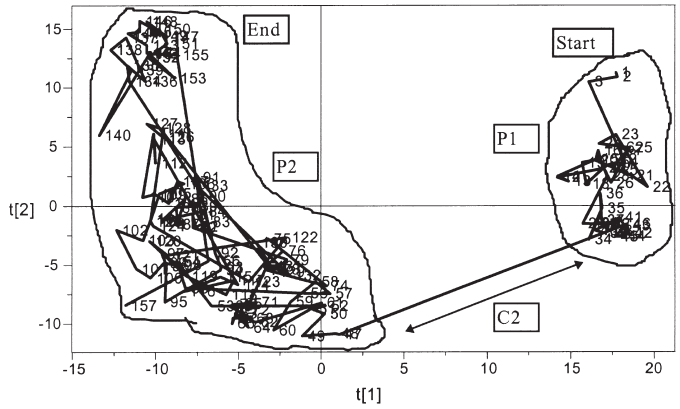

Figure 4. Scores plot for the two main principal componenets on second derivative pre-treated data collected with the flow cell.

In the first derivative pre-treated scores plot, Figure 3, the process change (C2) and all the characteristics which were detectable in the absobance data plot (Figure 3) could be detected.

For the second derivative pre-treated data the PCA, Figure 4, did not show as much structure as the PCA for absorbance and first derivative pre-treated data. The only structure clearly visible was the change between phase 1 and 2. The actual disturbance and the path back to normal conditions were not detected for the second derivative pre-treated data.

The PCA for the transmission probe data, Figures 5 and 6, were also able to resolve the two phases and the two changes in the process. During the experiment the probe probably clogged or a bubble was caught in the probe slit. This problem disappeared and the process fell back to the same track according to the score plot. During the same experiment, an infection occurred and was detected in the score plot prior to detection by the other on-line instruments. This shows the potential for scores plots in the qualitative analysis.

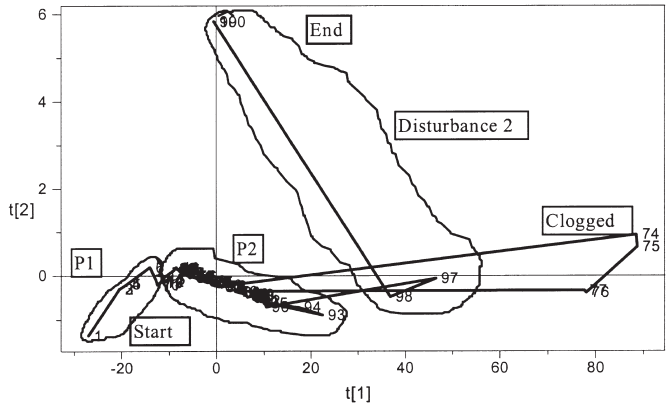

Figure 5. Scores plot for the two main principal componenets on absorbance data collected with the transmission probe.

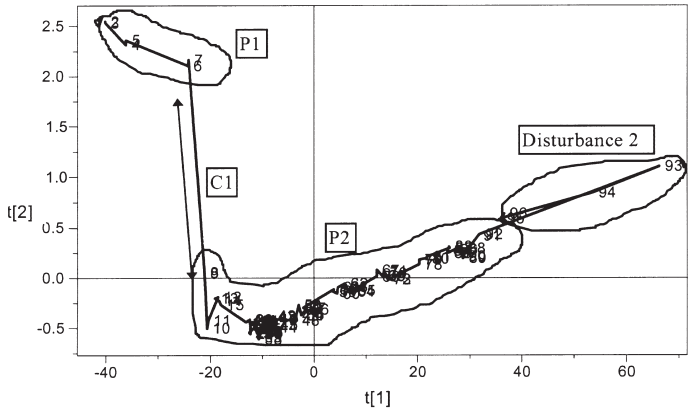

Figure 6. Scores plot for the two main principal componenets on absorbance data collected with the transmission probe. Spectra 74–77 represent the clogging has been aborted.

Flow-cell vs transmission probe

The disadvantage with the probe was that it clogged during the experiment. An additional disadvantage is that the calibrations are only valid for the same reactor dynamics and that the position of the probe should not be alternated during use as the flow through the slit should preferably be the same. By using the flow cell, the reactor dynamics may be alternated as long as the flow through the flow cell is the same as used during the calibration. For mammalian cell culture the by-pass system needed for the flow cell does not create any major changes in the processes as might be the case for bacterial fermentations.

Conclusion

The study shows that there is qualitative information in the spectra and that the information can be enhanced by mathematical pre-treatments and visualised by PCA. The qualitative picture can be used

for tracing and fault detection, or as an alarm system for the cultivation when it starts to deviate from the optimum track. Also, the possibility of monitoring and controlling the cultivation by relating the PCA to quality parameters has great potential for future development. The principal components can be related to the partial least square calibration to investigate which parameters are related to the principal components studied. In our case, these clear visible results helped us to convince sceptics that near infrared spectroscopy can be related to real process information.

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