Application of *in-line* near infrared spectroscopy to the control of industrial fermentation processes

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Introduction

Process quality control has become essential for both the agro-food and other industrial sectors. From an analytical perspective, this translates into a requirement for dependable, selective and sensitive analytical techniques. Such a requirement is particularly felt in the biotechnological sector where, in addition to some easily measurable physical and chemical parameters, several other crucial process variables such as biomass, substrates and metabolic products, ideally, should be monitored in real-time in order to maximise process yields and product quality. Modern fermentation technology increasingly relies on interactive process control which can only be optimally applied if a real-time, complete analytical characterisation of the process is available. A key requirement of any process monitoring technique applied to biotechnological processes is that it should not endanger process sterility: in this respect non-invasive analytical techniques which do not need to break the sterility barrier to obtain samples for off-line analysis have a tremendous advantage.

However, the current lack of suitable sensors means that the control of fermentation processes relies largely on the on-line measurement of physical parameters which relate only indirectly to the important biological and biochemical process variables. Real-time monitoring of such parameters is crucial to the optimisation of industrial fermentation processes because conventional off-line monitoring cannot adequately represent a dynamically-changing system. It is clear that any improvement in the response time of process monitoring is bound to translate into process improvements.

A spectroscopy-based approach is possible, in principle, given that the majority of the compounds of interest possess sufficiently differentiated spectroscopic features, but the inherent turbidity of fermentation broths prevents a direct *in-situ* determination by means of conventional UV/vis, or infrared (IR) techniques. As a result, the important biological and biochemical process variables are usually monitored by means of off-line techniques such as chromatography (HPLC, GC, etc.). These are time-consuming, require that samples be withdrawn and, generally, yield their results after a time-lag.

Against this background, near infrared (NIR) spectroscopy, coupled to suitable chemometric techniques, has considerable promise because it can combine real-time determination with non-invasive operation. An additional bonus is the elimination of the potentially toxic and/or environmentally- unfriendly reagents typically required by conventional analytical methods.

In order to demonstrate the applicability of NIR spectroscopy to the control of an industrial bioprocess, we have developed an NIR-based fermentation monitoring system capable of monitoring the concentration of biomass, growth substrates and metabolic products in two fermentations used to produce starter cultures for the industrial production of salami.

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Objectives

Our group has utilised NIR spectroscopy in the biotechnological field for several years[1–5] working, in particular, with anaerobic cultures of a homofermentative *Lactobacillus* strain. The work reported here is the ideal continuation of previous research in that it aims at evaluating NIR spectroscopy as a tool for the real-time monitoring of all relevant parameters of two aerobic fermentations based on agitated and aerated cultures of different microorganisms grown in complex cultivation media. Non-invasive monitoring was achieved by means of a sterilisable probe inserted into the fermenter.

Experimental

Acquisition of NIR data

The probe, fitted to the fermenter head-plate, acquired spectroscopic data in the *interactance* mode and was connected to the NIR instrument by means of an optical fibre bundle (Foss NIRSystems 6500). The probe slit was set to 1 mm, thus providing an optical path of 2 mm. The culture broth flowed across the optical path of the probe as a result of mechanical stirring of the culture and was completely exposed to the prevailing hydrodynamic conditions of the fermenter.

Fermentations

The fermenter utilised (BM 3000 CLP-Bioindustrie Mantovane) had a geometrical volume of two litres and was completely computer-controlled. Two microorgansims were utilised, one belonging to the genus *Staphylococcus*, and the other to the genus *Lactobacillus*. The same complex, glucose-based cultivation medium was used with both organisms. The following process variables were monitored: glucose (main C-source), lactic acid and acetic acid (main metabolites) and biomass.

Off-line analysis

The biomass concentration was determined as the dry weight of the culture, measured by the membrane filtration method (cellulose acetate filters, pore size 0.45 μm). The remaining components were determined by direct HPLC analysis of fermentation samples (column: Aminex HPX-87H).

Calibration and validation

NIR spectra were recorded in the 700–1800 nm region and were then transformed into second derivative spectra to reduce baseline offsets. About 170 samples were thus analysed for each culture.

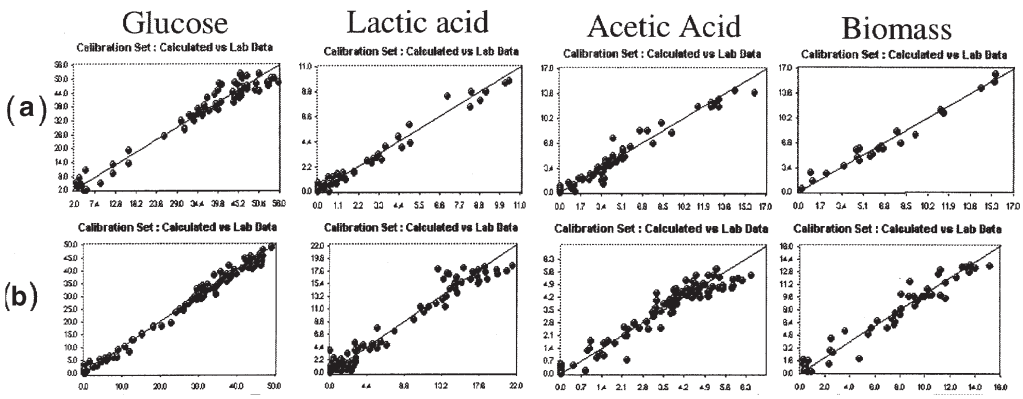

Figure 1. Calibration curves for (a) *Staphylococcus* and (b) *Lactobacillus*.

Table 1. Calibration and external validation parameters.

	Staphylococcus				*Lactobacillus*			
	Calibration		Ext. Validation		Calibration		Ext. Validation	
Glucose	Range R^2	0–58 gL^{-1} 0.9708	R^2 *SEP*	0.9679 2.9123	Range R^2	0–50 gL^{-1} 0.9883	R^2 *SEP*	0.9635 2.2730
Lactic acid	Range R^2	0–23 gL^{-1} 0.9381	R^2 *SEP*	0.9393 1.3669	Range R^2	0–22 gL^{-1} 0.9595	R^2 *SEP*	0.8926 1.8626
Acetic acid	Range R^2	0–19 gL^{-1} 0.9573	R^2 *SEP*	0.9010 0.6280	Range R^2	0–7 gL^{-1} 0.9488	R^2 *SEP*	0.8675 0.4804
Biomass	Range R^2	0–16 gL^{-1} 0.9506	R^2 *SEP*	0.9535 0.8532	Range R^2	0–16 gL^{-1} 0.9514	R^2 *SEP*	0.9578 0.5481

Calibration equations for the four components of interest were obtained by means of PLS regression using an iterative procedure that added the samples used for the *external validation* to the calibration database. The procedure was repeated until the required level of predictive capacity was achieved. The main statistical parameters of the calibration curves and the final *external validations* of all process parameters for both microorganisms are summarised in Figure 1 and Table 1.

Results and discussion

Effects of cultivation enviroment, agitation rate and airflow rate

A preliminary set of experiments was carried out to evaluate the influence of process conditions such as steam sterilisation, fouling by culture components and culture hydrodynamics on data acquisition by means of the immersion probe. Neither *in-situ* steam sterilisation nor fouling appeared to negatively influence probe performance. However, the system was negatively influenced by the air bubbles generated by air sparging and stirring of the culture, required by the aerobic nature of the fermentations being carried out. Stirring speed variations, in particular, were shown to determine major baseline shifts, irrespective of biomass concentration. In fact, it produced a marked signal change, presumably due to its effect on the population of air bubbles suspended in the culture fluid and had to be kept constant throughout the fermentation runs (Figure 2).

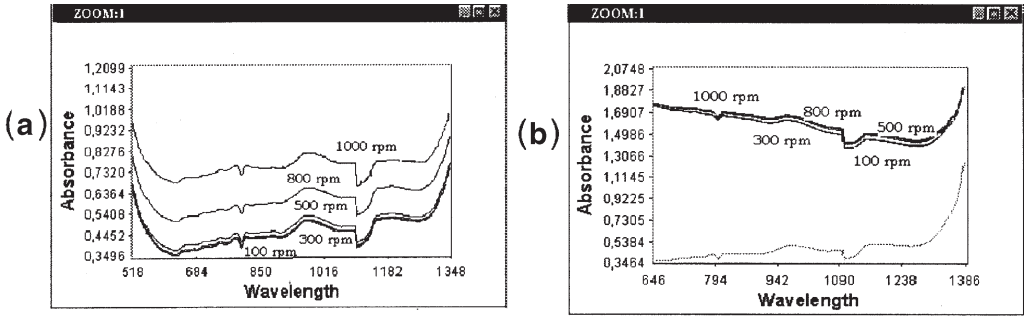

Figure 2. Stirring rate effects on spectrum baseline at (a) low and (b) high biomass concentration.

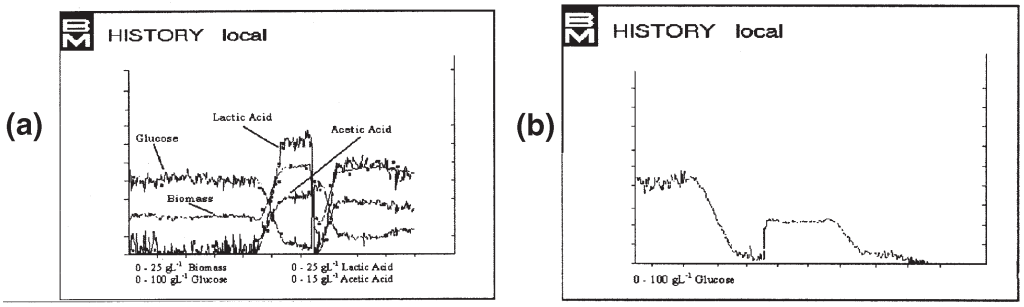

Figure 3. Automatic control of (a) repeated batch and (b) continuous fermentation processes.

Real-time, quantitative monitoring of fermentation parameters

Based on the encouraging results of the external validations for the biochemical parameters of interest, we set out to evaluate the applicability of *in-line* NIR spectroscopy to real-life fermentation monitoring and control. This was achieved thanks to the direct interfacing of the NIR instrumentation with the PC-based control system of the fermenter. The values of the relevant process parameters obtained by means of the NIR technique were thus transferred in real time to the fermenter control system where they were used for immediate numerical and graphical display and for the implementation of various cultivation strategies. As a result, both fed-batch and continuous fermentations employing the two microorganisms were carried out (Figure 3). Simultaneous off-line analysis confirmed the validity of the in-line NIR measurements, demonstrating the ability of this technique to monitor satisfactorily an entire process, while minimising operator interventions.

Conclusions

The NIR-based data acquisition system utilising a steam-sterilisable probe, immersed directly into the culture fluid, has been shown to be a viable alternative to traditional monitoring methods based on sample withdrawal and off-line analysis. The validity of the approach was confirmed even when applied to multicomponent, time-variable matrices such as fermentation broths with varying levels of turbidity. The elimination of sterility hazards connected to sampling is particularly relevant to biotechnological processes. Once the main task of setting up and validating the required calibration curves is completed, a one-probe non-invasive monitoring system is in place and only requires a suitable interfacing with process computers to allow real-time monitoring and control of fermentations. A tool is, therefore, available for the automatic control of fermentation processes on the basis of suitable process models and control strategies. Finally, it should be pointed out that the results reported here are relevant to the monitoring and control of several submerged processes, both inside and outside the scope of biotechnology.

References

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