Control of fermentations by means of on-line near infrared spectrometry

E. Dosi, G. Vaccari, A. L. Campi and G. Mantovani
Department of Chemistry, University of Ferrara, 44100 Ferrara, Italy.

A. Gonzaléz-Vara y R.
Department of Pharmaceutical Sciences, University of Bologna, 40126 Bologna, Italy.

A. Trilli
Bioindustrie Mantovane spa, 46040 Gazoldo Ippoliti, Mantova, Italy.

Introduction

Development and optimisation of fermentation processes are strongly dependent on accurate, real-time control of chemical and physical process variables. Computer control of such processes is in turn dependent upon continuous, automated data acquisition. This is at present only possible, using either heat-sterilisable probes, or non-invasive techniques, with physical and chemical parameters such as temperature, pH, dissolved gas (essential O_2 and CO_2) concentration, pressure, weight/volume, off-gas composition, redox potential, turbidity, fluorescence and a number of electrical properties of the culture that can be related to the concentration of biomass. For most chemical parameters the only practical solution is still off-line instrumental determination. The inherent delay between sampling and acquisition of data severely limits the potential for automated fermentation process control.

While several novel methods of *in situ* measurement of chemical species inside bioreactors are currently under development, a fundamental problem remains to be solved, i.e. the potentially large numbers of chemical parameters that would be desirable to measure simultaneously on-line. Approaches based on dedicated sensors, while solving specific analytical problems do not address this basic requirement of fermentation monitoring and control.

Near infrared (NIR) spectroscopy offers considerable promise in this respect, but only recently it has been considered as a possible tool of fermentation technology, perhaps surprisingly in consideration of its well-proven versatility, rapidity of response and non-invasivity.

Nishinari *et al.*[1] described on-line control of glucose concentration during starch hydrolysis, Cavinato *et al.*[2] used an optical-fibre NIR instrument to monitor the alcoholic fermentation, Picque *et al.*[3] reported monitoring of the alcoholic and the lactic acid fermentations, Brimmer and Hall[4] and Yano and Harata[5] described the use of NIR to monitor nutrient concentrations, Macaloney *et al.*[6] use NIR to monitor the glycerol fermentation, Hall[7] indicated direct NIR bioreactor interfacing as the way ahead in fermentation process monitoring and control.

Such an approach had already been advocated by the authors, Vaccari *et al.*,[8–10] as the solution of choice for monitoring glucose to lactic acid fermentation by *Lactobacillus casei* subs. *casei* DSM 20011. Calibration curves for glucose, lactic acid and biomass were obtained, and the

From Near Infrared Spectroscopy: The Future Waves

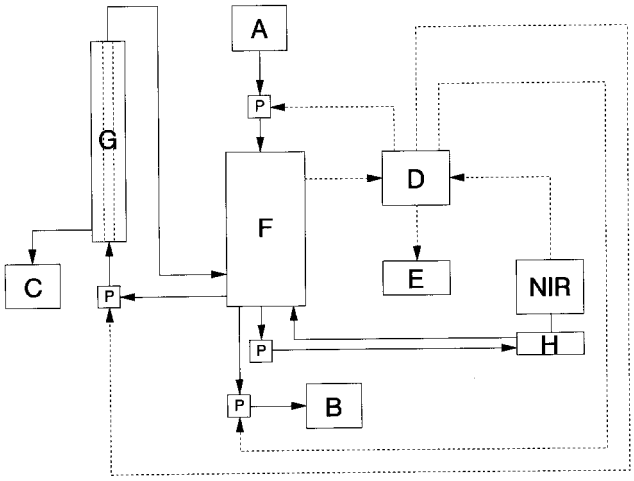

Figure 1. Scheme of connections between the NIR instrument and the bioreactor: (F) bioreactor vessel; (D) computer; (E) recorder; (H) NIR cell; (G) microfiltration unit; (P) pumps; (A) medium reservoir; (B) culture harvest; (C) permeate harvest.

time-course of the fermentation was successfully followed by interfacing the NIR instrument to the bioreactor. The logical development of such work, i.e. direct control of the fermentation by means of NIR interfacing, is reported here. To explore the capabilities of this approach, a dedicated software enabling on-line NIR data acquisition and reduction, and automated process management through feed addition, culture removal and/or product recovery by microfiltration was developed. A number of different fermentation techniques were tested and successfully implemented. A detailed discussion of the physiological aspects of the experiments is not included here, and will be the subject of a separate report.

Experimental methods and results

The experimental set-up, including the connection between the bioreactor, the NIR instrument, the feed addition and/or culture removal equipment, and the microfiltration unit, is schematically shown in Figure 1. The NIR instrument used was an InfraAlyzer 450 (Bran+Luebbe, Norderstedt, Germany) while the bioreactor model BM-PPS3 and the microfiltration unit were supplied by Bioindustrie Mantovane, Gazoldo Ippoliti, Mantova, Italy. A view of the whole set-up is shown in Figure 2.

Culture broth was continuously circulated through an external loop including the NIR measuring cell. Data acquisition and reduction was carried out every three minutes. According to the fermentation strategy adopted, fresh medium was added to the culture, the microfiltration module was activated, or excess culture was removed from the vessel, in a completely automatic fashion.

A total of six different fermentation strategies were explored, each starting with a conventional batch process. Transition from batch mode to automatic control mode was triggered by pre-defined criteria, e.g. the degree of substrate conversion, the concentration of biomass achieved etc. Figure 3 shows an example of the time-course of the batch phase, as represented on the bioreactor computer screen. Cultivation medium and conditions were the same as reported by Vaccari *et al.*[8–10]

From Near Infrared Spectroscopy: The Future Waves

Figure 2. View of experimental set-up including bioreactor, process computer, microfiltration module and NIR instrument.

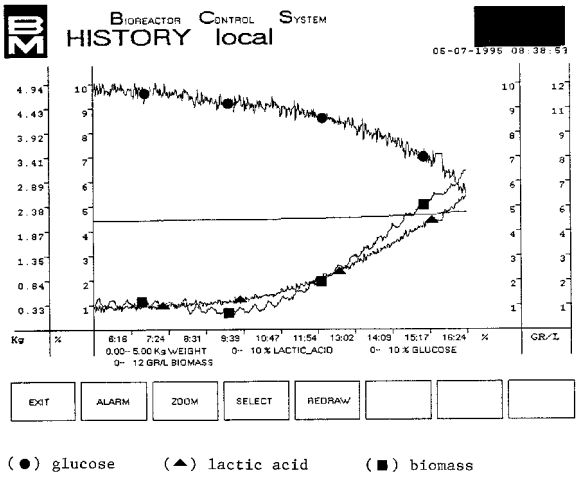

Figure 3. Computer graphical representation of process data during batch phase of experiments. (●) glucose; (▲) lactic acid and (■) biomass.

Case 1

A repeated-batch fermentation technique was adopted, where culture removal was triggered by the achievement of a pre-defined concentration of lactic acid. Culture volume (weight) was reduced to a pre-determined value and then fresh medium was rapidly added to reach the set cultivation volume. Culture discharge and re-fill were automatically managed by the bioreactor

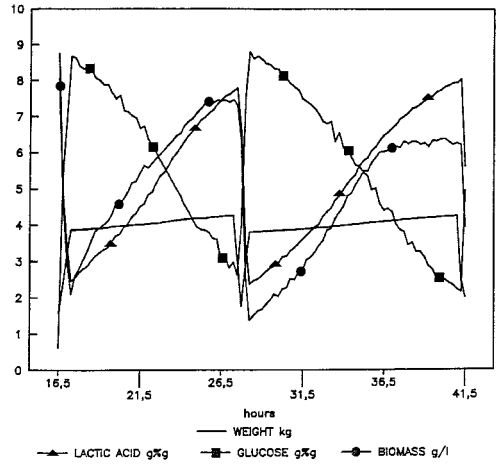

Figure 4. Case 1.

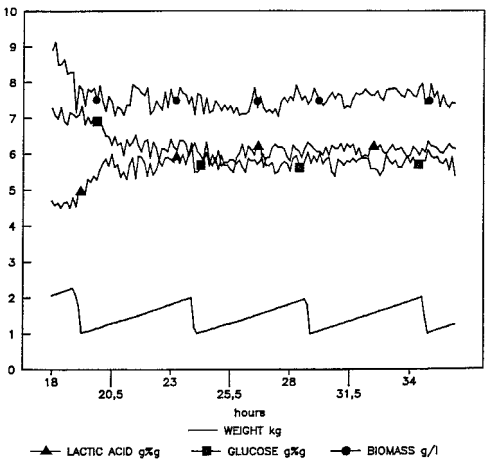

Figure 5. Case 2.

weight control system. Figure 4 shows the results of the first two cycles relative to series of consecutive batch cycles.

Case 2

Conventional repeated fed-batch fermentation with pre-set values of initial volume, final volume, and feed rate. NIR instrument was used only to monitor substrate, product and biomass concentration. From the results obtained it was observed that the time-course of the three process variables is dependent on initial conditions. Figure 5 shows a sample of three fed-batch cycles.

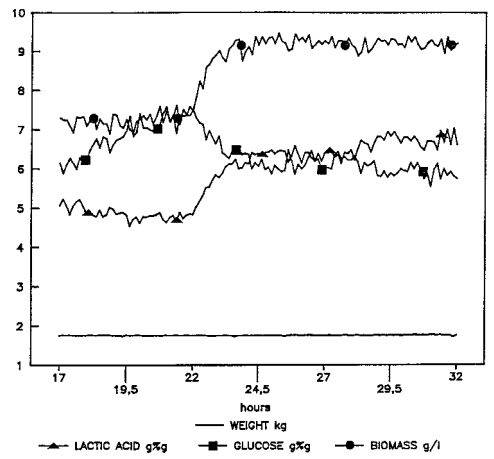

Figure 6. Case 3.

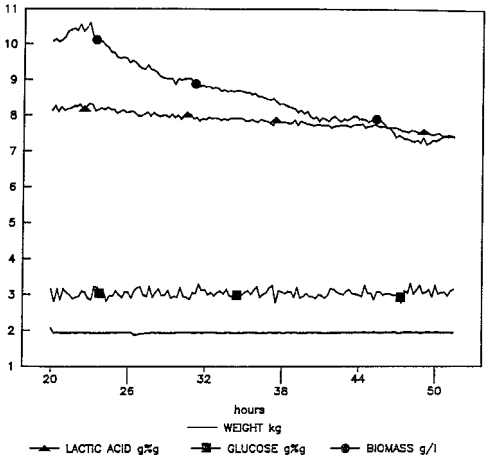

Figure 7. Case 4.

From Near Infrared Spectroscopy: The Future Waves

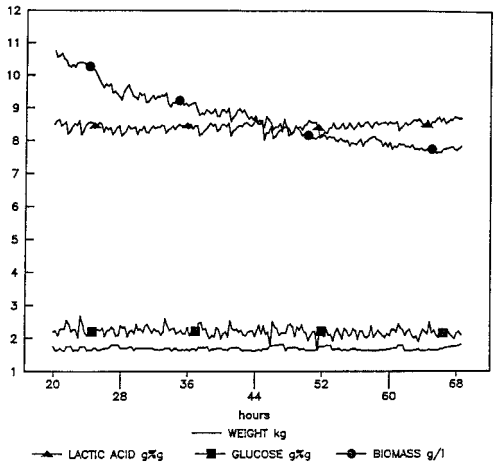

Figure 8. Case 5.

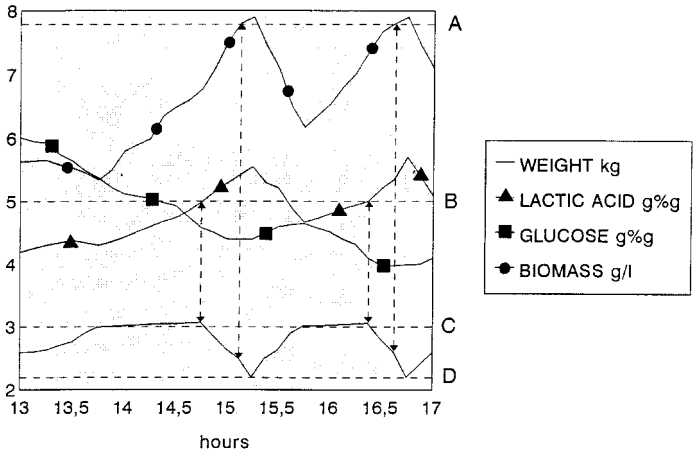

Figure 9. Case 6.

Case 3

Continuous flow cultivation with biomass concentration as the controlled variable. Essentially it consists of a turbidostatic operation at constant volume where medium flow is modulated to maintain biomass concentration at a pre-set value. Constant volume (weight) was maintained by the bioreactor weight control system acting on the discharge pump. Figure 6 shows the time-course of an experiment where biomass concentration was first set to 7 g L^{-1}, and then to 9 g L^{-1}. The resulting medium flow rate required to keep the biomass at the set value was obviously different in the two cases.

Case 4

The experiment was analogous to that of case 3, but glucose concentration was the controlled variable. The set value was 3 g%g. A progressive decrease of the biomass concentration, accompanied by a decrease of the medium flow rate, was noted. However, lactic acid concentration remained fairly constant (Figure 7).

Case 5

In this experiment an attempt was made to maintain both the glucose and the lactic acid concentration constant at pre-set values. Glucose level (2 g%g) was maintained by automatic fresh medium addition, while lactic acid level (8.5 g%g) was controlled by culture removal. In analogy to what was observed in case 4, a progressive decrease of biomass concentration and medium flow rate was observed (Figure 8).

Case 6

Essentially repeated-batch fermentation with periodic product removal by microfiltration and periodic removal of excess biomass. When a pre-set value of lactic acid concentration (B) was exceeded, the microfiltration module was activated. As a result biomass concentration increased. When the biomass threshold (A) was reached, culture was automatically discharged until a pre-set volume (D) was left inside the bioreactor. At this point automatic weight control took over to reconstitute the culture volume to the pre-set working level (C) by adding fresh medium. A new cycle was triggered by the lactic acid concentration reaching the (B) threshold (Figure 9).

Conclusions

The results shown clearly demonstrate that direct NIR interfacing to a suitably automated fermentation system allows a number of different fermentation strategies to be implemented successfully. While a number of technical aspects, e.g. the use of *in situ* probes vs external circulation loops, probe geometry etc., need to be addressed to allow a generalised application of bioreactor-NIR interfacing, a considerable application potential can already be exploited in well-identified production processes as well as in process development and optimisation.

References

1. K. Nishinari, R.K. Cho and M. Iwamoto, *Starch/Starke* **41(3),** 110 (1989).
2. A.G. Cavinato, D.M. Mayes, Z. Ge and J.B. Callis, *Anal. Chem.* **62,** 1977 (1990).
3. D. Picque, D. Lefier, R. Grappin and G. Corrieu, *Analytica Chimica Acta* **279,** 67 (1993).
4. P.J. Brimmer and J.W. Hall, *Canadian J. of Applied Spectroscopy* **38(6),** 155 (1993).
5. T. Yano and M. Harata, *J. of Fermentation and Bioengineering* **77(6),** 659 (1994).
6. G. Macaloney, J.W. Hall, M.J. Rollins, I. Draper, B.G. Thompson and B. McNeil, *Biotech. Technique* **8(4),** 281 (1994).
7. J.W. Hall, Paper presented at 6th International Conference on Near Infrared Spectroscopy. Lorne, Victoria, Australia, 18th–22nd April (1994).
8. G. Vaccari, E. Dosi, A.L. Campi and G. Mantovani, *Zuckerindustrie* **118,** 266 (1993).
9. G. Vaccari, E. Dosi, A.L. Campi, A. Gonzaléz-Vara y R., D. Matteuzzi and G. Mantovani, *Biotechnology and Bioengineering* **43,** 913 (1994).
10. G. Vaccari, E. Dosi, A.L. Campi and G. Mantovani, *Zuckerindustrie* **120,** 225 (1995).

From Near Infrared Spectroscopy: The Future Waves