Applications of near infrared spectroscopy to fermentation process analysis

I.K. Brookes, B.N. Gedge and S.V. Hammond

Pfizer International, Sandwich, Kent, UK.

Introduction

Since 1988 Pfizer have been utilising near infrared (NIR) spectroscopy for monitoring fermentation processes. Initial applications in bio-reactor monitoring were reported at the 4th International NIR Spectroscopy Conference.[1] Progress has now taken the technique into broth recovery and the testing of bulk fermentation materials and formulated products.

Experimental

Safety

NIR analysis is in itself non-hazardous. Any hazard will be associated with the sample. Appropriate precautions must be taken when handling samples.

Apparatus

Laboratory investigations were performed using an NIRSystems 6500 scanning monochromator (NIRSystems, Silver Spring, MD). The instrument was equipped with a sample transport module for all at-line work. A static sample cup module was used for flow cell work and a remote reflectance fibre optic probe for non-invasive work. The plant scale investigations used a standard NIRSystems "Interactance" probe (part no. NR-6640) connected to an "on-line" NR5000 spectrophotometer (NIRSystems). The software used for this work was the NSAS package provided with the instrument.

Procedure for at-line calibration

Calibration was carried out by analysing in replicate 50 to 60 samples using standard analytical methods and collecting their reflectance spectra in quartz cuvettes. The samples were selected to be representative of all the variations likely to be encountered; process changes, medium formulation and the natural variation in substrates. Several similar calibrations were produced for each parameter and subjected to a validation process resulting in selection of the most robust calibration for actual use.

From Near Infrared Spectroscopy: The Future Waves
© IM Publications Open LLP 1996

Discussion

Problem definition

Maximum fermentation performance requires tight control of biological state. The input and effect of nutrients must be balanced with the uptake rates, respiration demands, power input and mixing efficiency of the bio-reactor.

Sensors for rapid analysis have promised much but have been found to lack robustness in complex industrial fermentation media. Often, on-line measurements have been limited to off gas monitoring of respiration, therefore Pfizer have a strong desire to develop rapid at-line analytical methods with the potential to be transferred for on-line use.

In the recovery and product isolation streams, in-process assays are often laborious and too slow to provide good control.

Why NIR?

NIR has three key attributes for performing these measurements; no sample preparation is required, one scan can provide multiple parameters and sample presentation is extremely versatile, in that various modes of reflectance measurements can be made along with conventional transmission spectroscopy. Both of these approaches can also be effected using remote fibre optics.

All of the above approaches have been investigated, scanning broth contained in quartz cuvettes directly in reflectance, broth recovery solutions in transmission and fibre optic probes either immersed in broth or remotely scanning through a reactor sight glass.

Methodology

Calibration is performed by statistical comparison of reference method data and NIR spectral data. A good calibration will result in the correlation plot seen in Figure 1.

The calibration process is highly mathematical and an abstract process and, as such, requires very careful validation. It must be demonstrated that the calibration equation developed for one set of samples is applicable for all further samples. This is achieved by collecting a large additional set of sample spectra and testing the ability of the calibration to accurately predict the concentrations of the required constituent.

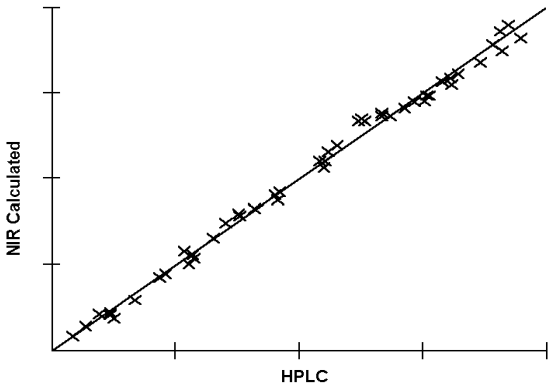

Figure 1. Correlation plot for at-line antibiotic measurement. HPLC data against NIR absorbance at 1690 nm, the C–H aromatic overtone. Reproduced from Reference 1 with permission ©Ian Michael Publications 1992.

From Near Infrared Spectroscopy: The Future Waves
© IM Publications Open LLP 1996

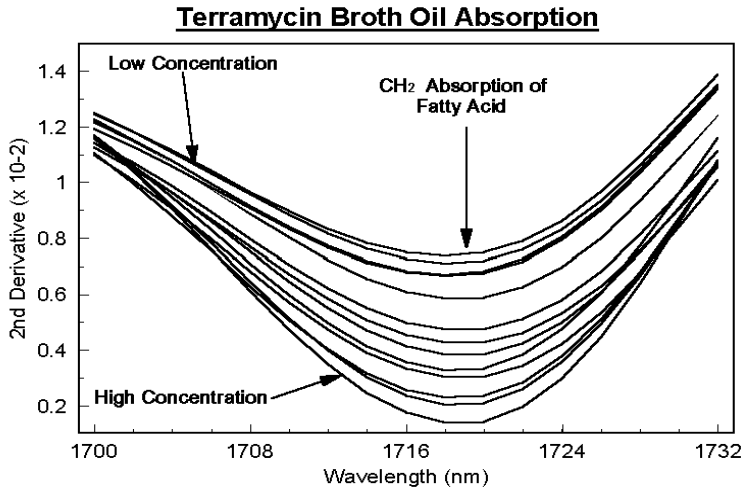

Figure 2. Typical CH_2 absorption of fatty acids.

A key ingredient for success in this process is to ensure that the wavelengths used for calibration are selective for the ingredient to be measured, e.g. at 1720 nm a characteristic CH_2 absorption of fatty acids is seen (see Figure 2) which provides a selective wavelength for measuring vegetable oil substrates.

Example applications

Fermenter control

For one of Pfizer's antibiotic fermentations, selective absorptions were identified for three parameters, as shown in Table 1. A reflectance spectrum of a broth is obtained by tipping neat sample into a quartz cuvette; the spectrum can be simultaneously applied to separate calibrations for the three parameters, generating a set of three results, in about two minutes. By conventional assay methods two hours would be necessary to produce the same assay results.

The success of these at-line applications led to investigations of the on-line mode. The first system tried used a loop and flow-cell (Figure 3). Very good spectra and calibrations were obtained but the pump was a weak sterility point and contamination tended to occur within a few days.

A second approach was to non-invasively scan the broth through the sight glass of the reactor. This approach was also successful and avoided the contamination risk.

Table 1. NIR absorbances of functional groups used to construct calibrations.

Constituent	NIR absorbance
Fat	1716 nm, the CH_2 overtone absorption of fatty acids
Cell growth	2394 nm, the CH_2 combination absorption of techoic acids
Antibiotic	1660 nm, the C–H aromatic overtone

From Near Infrared Spectroscopy: The Future Waves
© IM Publications Open LLP 1996

The direct insertion of a fibre optic probe into a production reactor was investigated (see Figure 4). Data collection had to be optimised to overcome the inherent noise of fibres above 2100 nm. This was achieved by collecting four lots of 50 scans (with a reference in between each set) and averaging. Once this was done calibration was successfully achieved (see Figure 5).

The ability of the two direct approaches to cope with a fast moving sample, containing a high density of air bubbles, was testimony to the versatility of NIR spectroscopy.

The use of NIR to rapidly measure soluble substrates, such as glucose, was found to be possible with broth filtrates, but proved difficult on whole-broth, as the characteristic absorptions of glucose are masked by the large amount of natural carbohydrate in the broth (see Figure 6).

Broth processing/product recovery

The success with fermenter control led to the investigation of the technique in broth processing and product recovery. For Terramycin the recovery process involves solubilising the product in acid solution, which is then filtered to remove the broth solids. A number of clean-up stages are then carried out using selective precipitation and filtration (Figure 7). A single calibration, (Figure 8) based on the transmission spectra of the filtrates from each stage, has proved an accurate and robust control method, in spite of the background chemical composition of filtrates being different.

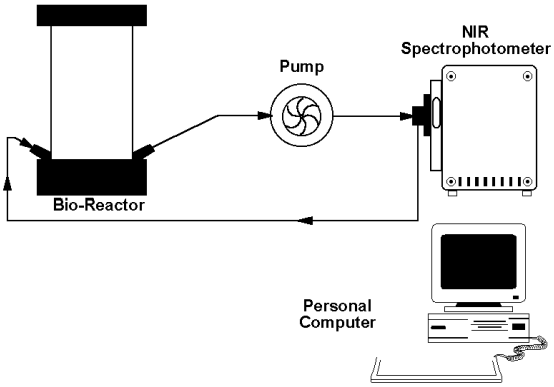

Figure 3. Illustration of the "flow cell loop" on-line system (Adapted from Reference 1).

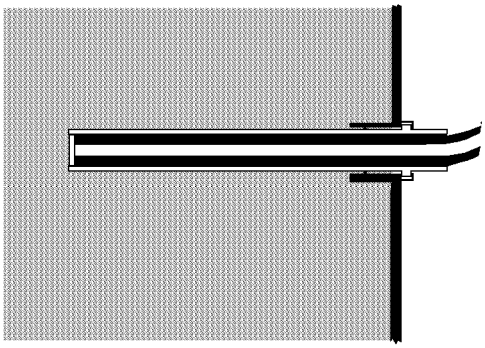

Figure 4. Direct insertion of fibre optic probe.

From Near Infrared Spectroscopy: The Future Waves
© IM Publications Open LLP 1996

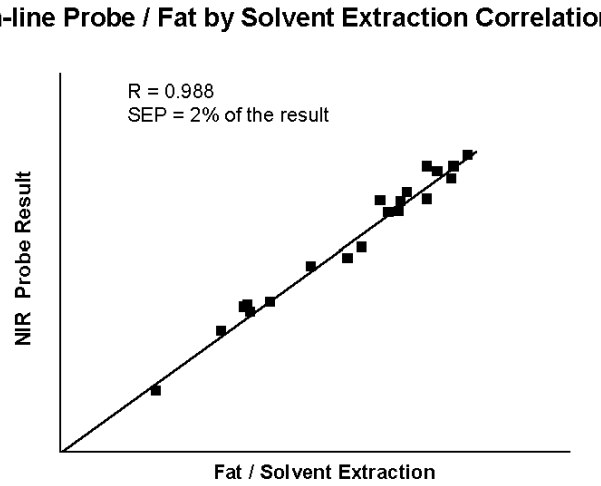

Figure 5. Residual fat by laboratory solvent extraction and on-line NIR prediction.

This robustness comes from the very selective wavelengths for the aromatic content of the molecule (Figure 9) and the microfiltration which removes all light scattering by the sample.

Two key lessons were learnt from this work. That calibration with different sample matrices is possible if you focus multivariate calibration on a common specific absorption. Microfiltered liquid samples can produce excellent calibrations for low levels (0.2%) of constituent.

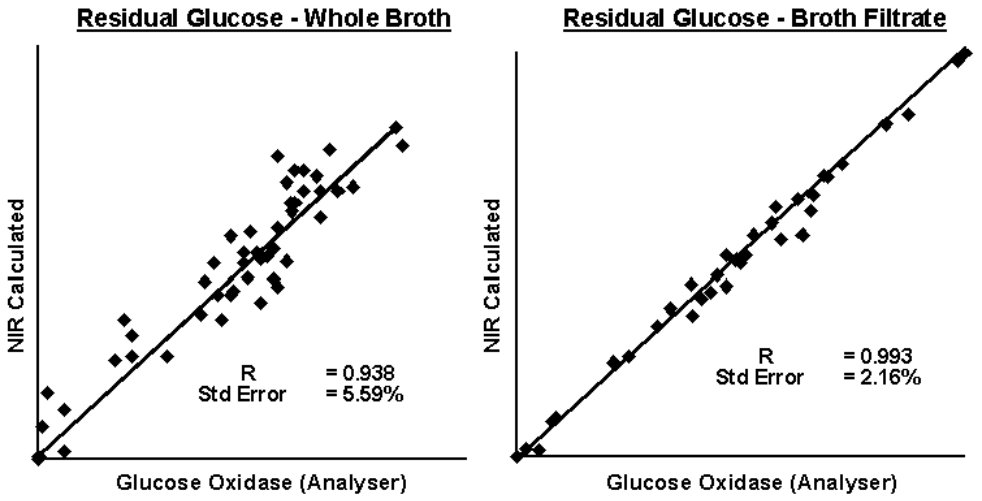

Figure 6. The effect of broth solids on glucose measurements.

From Near Infrared Spectroscopy: The Future Waves
© IM Publications Open LLP 1996

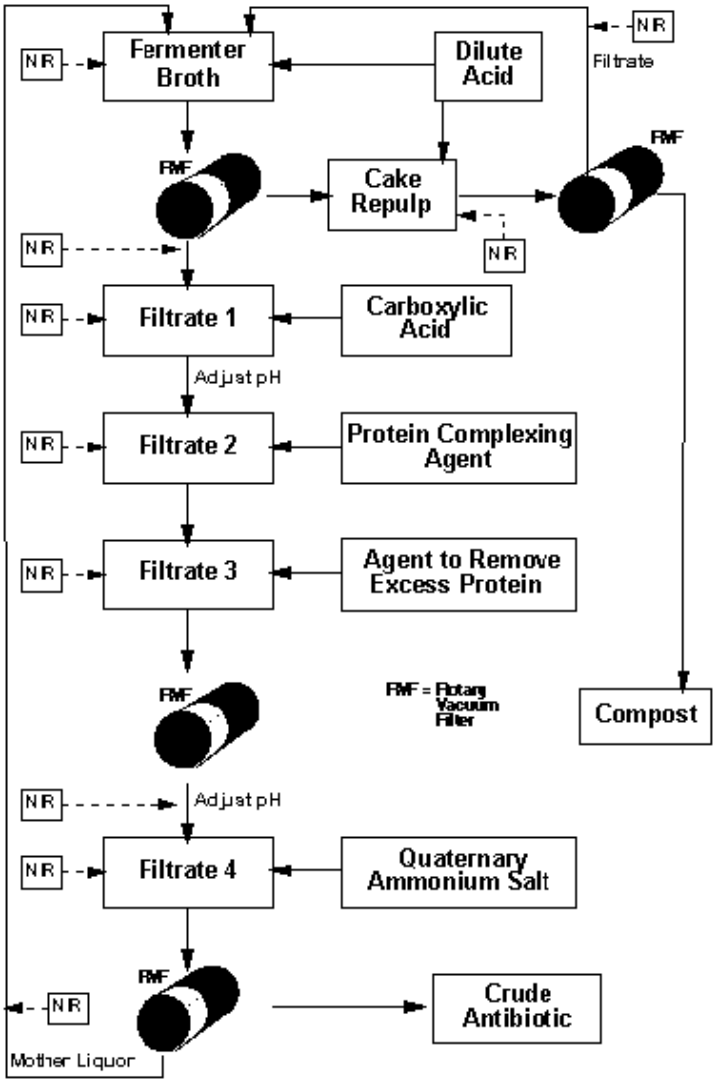

Figure 7. Product recovery streams.

Bulk product drying

Rapid NIR measurement of the water content of a spray dried feed (Figure 10) allows better control of this continuous process.

Finished product testing

The use of NIR has now progressed into the testing of the final product. Terramycin is blended into a lactose carrier at 5% and sold as a soluble powder. The potency of these powders is now determined using the NIR calibration shown in Figure 11.

From Near Infrared Spectroscopy: The Future Waves
© IM Publications Open LLP 1996

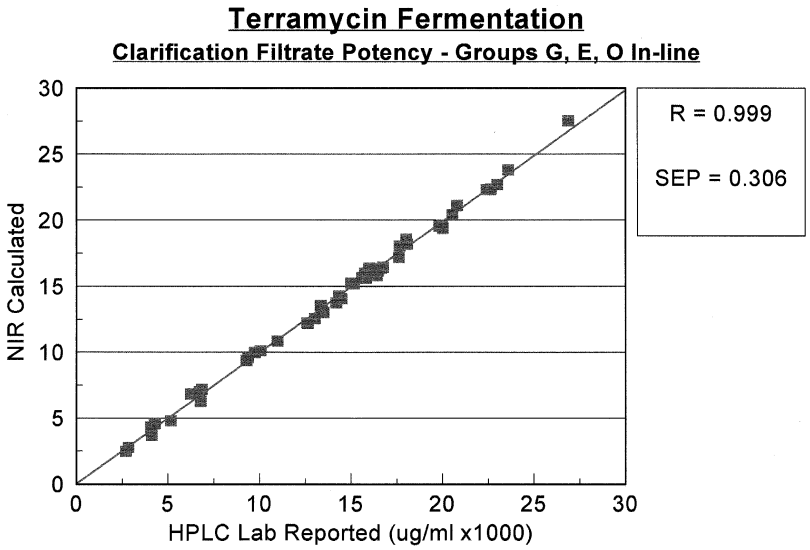

Figure 8. NIR predicted versus HPLC analysis of broth filtrates.

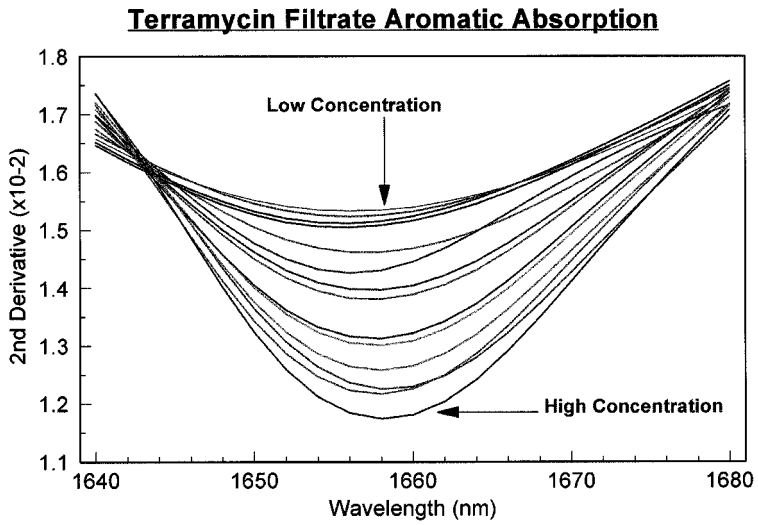

Figure 9. Typical aromatic C–H absorption of the antibiotic in the broth.

Benefits

Assay equivalence

For an NIR assay to be effective, it must provide the plant manager with data he is used to seeing, i.e. equivalent to the reference assay used for calibration. In Table 2, the NIR method is

From Near Infrared Spectroscopy: The Future Waves
© IM Publications Open LLP 1996

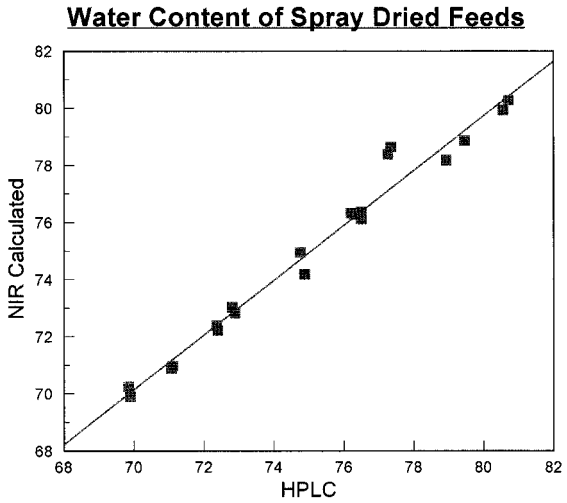

Figure 10. NIR predicted versus loss on drying for a spray drier feed.

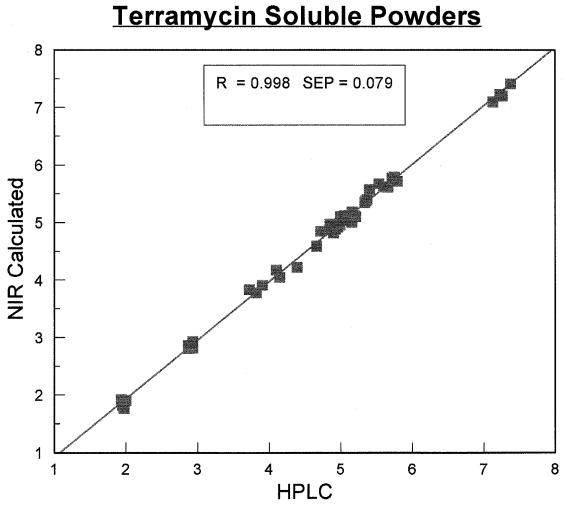

Figure 11. NIR predicted versus HPLC for commercial Terramycin soluble powder product.

compared with the reference assays used and shows improvements against crude non-selective assays and near equivalence to selective separative assays such as HPLC.

Benefits-basis

a) Tighter process control, based on faster analysis giving improved process consistency and performance.
b) Greater process knowledge—More frequent assays reveal subtle changes in correlated parameters, e.g. residual substrate and respiration rate.

From Near Infrared Spectroscopy: The Future Waves
© IM Publications Open LLP 1996

Table 2. Equivalence of the NIR method relative to the reference assay.

Constituent	Reference assay	*SE* of reference methods	*R*	*SEP* of NIR method
Fat	Solvent extraction	9.1	0.984	4.1
Cell growth	Dry cell weight	10.4	0.975	6.2
Oxytetracycline broth	HPLC	2.0	0.995	2.2
Oxytetracycline recovery	HPLC	1.0	0.99	1.1
Oxytetracycline product	HPLC	0.024	0.994	0.025

All numbers have been normalised to percentages.

c) Improved product quality assurance—NIR testing can provide an additional benefit with product analysis, in that a rapid qualitative "conformity" test can be combined with quantitative measurements to provide a consistency test for the product.
d) Reduced costs—NIR is very efficient. The removal of sample preparation provides low assay times with reduced labour input. Faster analysis can also reduce inventory of the finished product.
e) Multiple applications on the same instrument—The same NIR instrument can be applied to all stages of the process, from raw materials, bulk refining through to finished product.

References

1. S.V. Hammond, "NIR Analysis of Antibiotic Fermentation", in *Making light Work: Advances in Near Infrared Spectroscopy*, Ed by I. Murray and I.A. Cowe, VCH, Weinheim, pp. 584–589 (1992).

From Near Infrared Spectroscopy: The Future Waves
© IM Publications Open LLP 1996