Application Note

KEIT spectrometers

Real-time analyser for in-process monitoring of **industrial fermentation** and **cell culture** processes

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Abstract

The IRmadilloDiamond™, a robust, process FTIR spectrometer, is used to monitor two different fermentation processes – a classical sucrose-yeast fermentation and an industrial bacterial fermentation. The production of six different chemical species was monitored in real time with concentrations ranging from 0.1 to 25 g L^{-1} using chemometric methodology.

It is shown how the in-process analyser can be used to directly improve the efficiency of biochemical reactions by providing early indication of process failure and enabling real-time control.

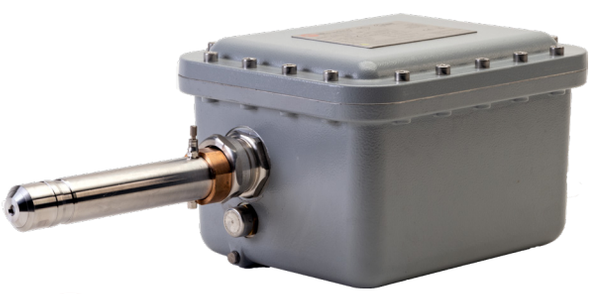
Introduction

There are many different ways that real-time process analytical technology (PAT) can be used in bioprocesses, for example:

- Monitoring the concentrations of feedstocks and using them for closed-loop control of fed-batch processes (for example, glucose in either CHO cell culture or many fermentation processes)
- Spotting contamination markers (such as lactic and acetic acid) early and either rectifying or aborting the batch in a cost-effective way
- Running the process at a much higher production rate where the product is toxic to the organism (ex., solvent concentration in acetone-butanol-ethanol [ABE] fermentations)

Spectroscopic PAT techniques such as Raman, Fourier transform infrared (FTIR) and near infrared (NIR) are all potentially powerful monitoring tools. They can directly detect different species simultaneously and can cope with a range of concentrations.

However, conventional spectroscopic techniques can suffer from fragility (in the case of traditional FTIR instruments), or low sensitivity and specificity (in the case of NIR instruments) and/or poor repeatability and performance drift (i.e., Raman).



IRmadilloDiamond™

Here we present the use of the diamond-probe IRmadillo, a robust and vibration-resistant FTIR spectrometer, to monitor fermentation processes. The process analyser has monitored fermentations of sucrose as well as commercial industrial fermentations.

Key Words

- Fermentation
- Cell culture
- Sugars
- Ethanol
- Closed-loop control
- Biochemical reactions
- On-line and in-line process monitoring

Features & Benefits

- Powerful mid-infrared spectral analysis
- Solid-state optical design
- Compact analyser
- Real-time concentration results
- Low maintenance
- Multi-component analysis

Experimental

The IRmadilloDiamond (IRmadillo) was either incorporated directly into a fermentation vessel through a side port, or placed in-line into a multi-stage process using a flow cell and peristaltic pumps. A background scan was taken for 30 min prior to immersing the probe in any liquid. Spectra were acquired over 120 s between 900 – 1900 cm^{-1}.

Chemometric models were built using partial least squares (PLS) and support vector regression (SVR) methodology.

Results and discussion

Figure 1 shows the traces for predictions of a simple yeast-based fermentation over approximately 32 hrs. The IRmadillo simultaneously tracks the concentrations of sugars (ranging from 0 – 110 g L^{-1}), ethanol (0 – 40 g L^{-1}), glycerol (0 – 5 g L^{-1}), as well as acetic and lactic acids (< 1000 ppm).

The independent monitoring of fructose, glucose and sucrose demonstrates the power of FTIR. Since sugars all have very similar structures, it can be difficult to differentiate between them. NIR in particular struggles to achieve this, and although off-line sampling can measure them independently, it is costly and slow.

Moreover, the spectrometer simultaneously measures the concentration of glycerol, and very low concentration acids. These can provide an early indication that the process is failing. Early detection of a failed batch is critical to improving production efficiencies and optimising cost savings.

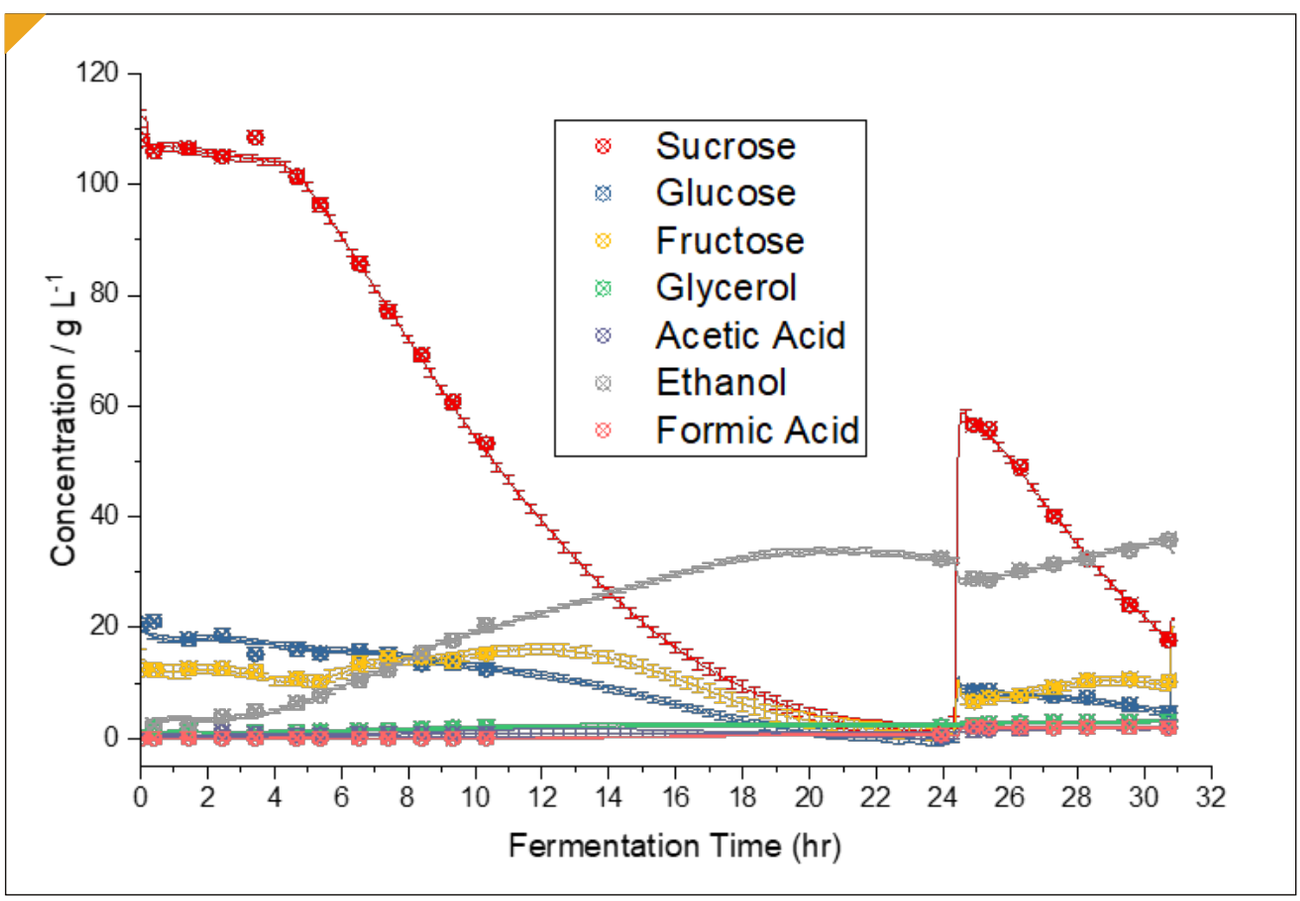


Figure 1: Plot of chemicals monitored by the IRmadillo during a fermentation process over 32 hrs clearly showing ability of the instrument to monitor multiple species simultaneously (sugars, acids, and alcohols).

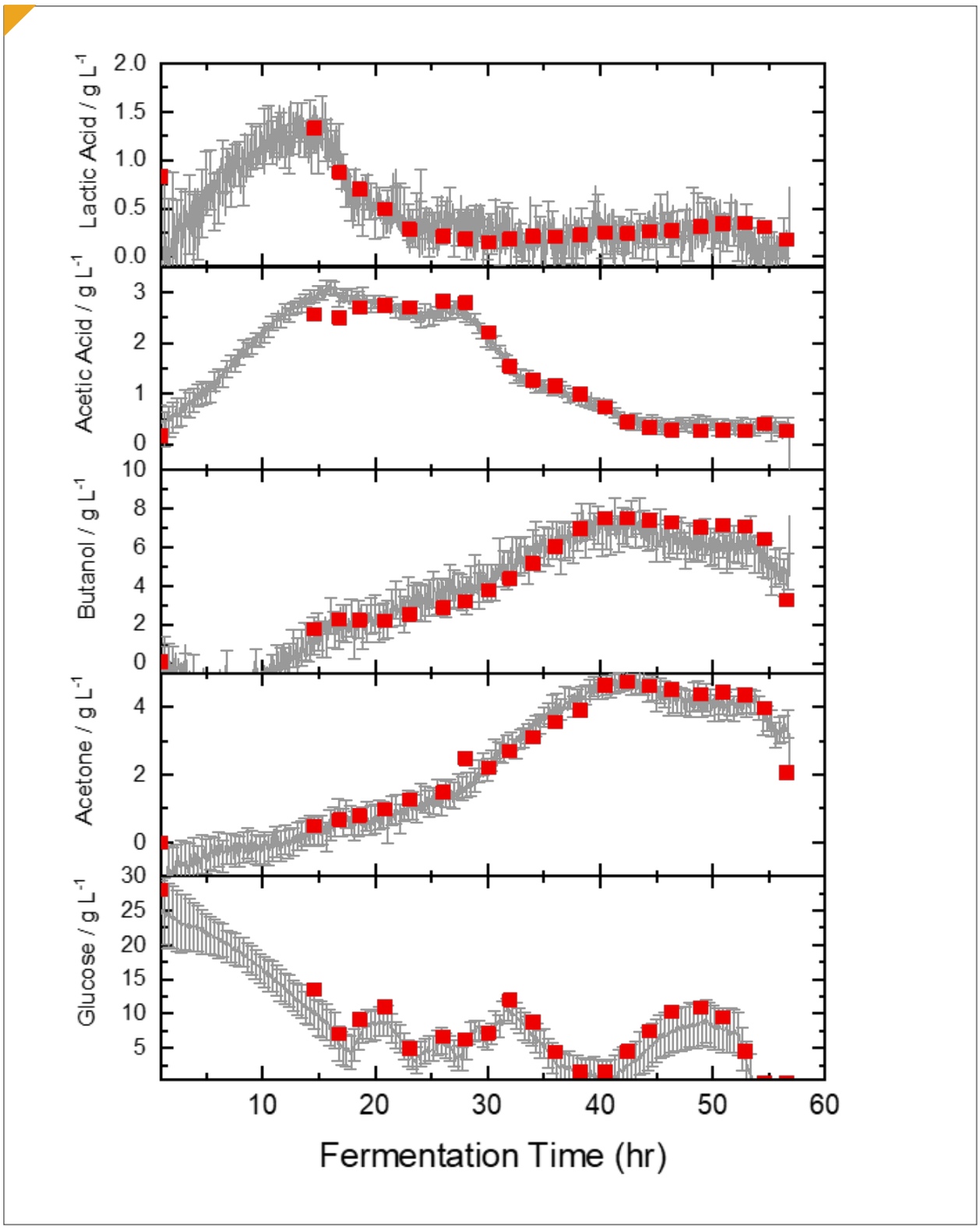


Figure 2: Prediction of reaction constituents throughout an ABE fermentation process with reference values from HPLC. The IRmadillo can be used for in-process PAT to effectively monitor a range of biochemical reactions for industrial applications.

Industrial fermentations cover a wide range of feedstocks and products, and are no longer limited to solely ethanol production.

Figure 2 shows the measurements of an ABE fermentation from an industrial manufacturer. This process uses frequent dosings of glucose and was aiming to use on-line PAT to achieve a constant glucose concentration through closed-loop control.

It is obvious that the glucose concentration with their standard approach fluctuates between 5 and 15 g L^{-1} and is easily monitored using the IRmadillo. The acetone and butanol concentrations can also be accurately measured, as well as the presence of organic acids (lactic and acetic in this case).

This shows that the IRmadillo is a suitable analyser for all types of bioprocesses regardless of the type of biomass and final product.

The IRmadillo is capable of monitoring almost any molecule, for example:

- Sugars
- Organic acids
- Alcohols (both mono-alcohols such as butanol and ethanol, but also di-ols and tri-ols such as glycerol)
- Proteins
- Amino acids
- Urea and ammonia
- Anions such as sulphates and sulphites, phosphates and phosphites, nitrates and nitrites

Conclusions

If the molecule has a covalent bond then the IRmadillo can probably detect and measure it. These results show that the IRmadillo can be effectively used to monitor different types of fermentations. Both yeast- and bacterial-based fermentations can easily be monitored, with the probe inserted either directly into the fermentation vessel or via a flow cell.

The IRmadillo allows the concentration monitoring of multiple different chemical species, with many different components being demonstrated here, over several orders of magnitude of concentration ranges.

This work shows that the application of PAT through the introduction of the IRmadillo into fermentations can help to reduce waste and improve efficiency, ultimately cutting costs.

What about sterilisation?

The IRmadilloDiamond's probe has been specifically designed to withstand both clean-in-place (CIP) and sterilise-in-place (SIP) protocols. The diamond ATR element can survive a pH range of 0 to 14. The probe has been tested up to 130°C and 20 barg pressure.

This means that once the probe has been installed it doesn't need to be removed for cleaning or sterilising, and can be considered a permanent installation.

NB: The IRmadilloDiamond has a fixed probe that is only available in 38 mm diameter. For installation into your process, Keit can provide a suitable port or flange for fixing onto your process vessel.

Interested in finding out more?

Visit our website to read more about the technical details of our instrument and other applications.

Contact us and let us know more about your process monitoring and what you'd like to measure in real time.

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IRmadillo™