

# Industrial Biotechnology

## Improving Production with the IRmadillo

### What is it?

The IRmadillo is a process analyser based on infrared light. It works by using a technique called FTIR spectroscopy to monitor the concentration of different chemicals in real time.

What makes it different to other analysers is that it's built to last. Not only last, but it's built to survive and built to perform in any environment.

There are no moving parts, and the light source inside of it runs at a low power – meaning it won't burn out mid-way through your process!

The lack of moving parts makes it very stable, so you can fit it and forget about it.

Health checks and maintenance can be done once a year.

### What's the point? Why should I use it?

Good questions! The point is that the IRmadillo monitors what's going on in your process as your feedstock is converted into product, in real time.

There are three main ways you can benefit from better fermentation and cell culture monitoring with the IRmadillo:

#### ■ Improve your yield

By having a trustworthy real-time measurement of the process you can run closer to maximum operating limits (i.e. keep glucose levels constant in a fed-batch process, or higher levels of toxic compounds – such as butanol in an ABE fermentation – during continuous processes).

#### ■ Improve your capacity

Identify the exact moment when a batch process has consumed its feedstock and reached optimum product level, then stop the process. In some cases (i.e., bioethanol) the organism will actually start consuming the product, so identifying the moment your process is complete can save substantial amounts of money at scale.

#### ■ Rectify Problems

The IRmadillo lets you see exactly what's going on in your process, so, if something does go wrong you have time to fix it and not lose the batch (i.e. if lactic acid rises early on you can spot an infection and either rectify it or abort the batch early enough to minimise costs).



### How do I use it?

The IRmadillo is a versatile process analyser based on FTIR spectroscopy. This means you can use it for concentration monitoring (quantitative analysis) or for condition monitoring (qualitative analysis).

#### ■ Quantitative analysis

The IRmadillo will output the concentration for each different chemical present in your process that it's been calibrated to detect. Example chemicals are:

- 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

There's no maximum concentration, and the scales can be adjusted depending on the chemical of interest. So you can monitor sugars in % terms while keeping an eye on ppm level organic acids at the same time.

#### ■ Qualitative analysis

The IRmadillo will give you readings such as "within specification", "contamination spotted", or "fermentation stressed" for example. The calibration

### What about cleaning? And sterilisation?

The IRmadilloDiamond model was specifically designed with clean-in-place (CIP) and sterilise-in-place (SIP) processes in mind. The probe can survive pH 0 to 14, and temperatures up to 130°C (provided you turn off the emitter – easy to do from the software). It will cope with pressures of up to 20 bar – no problem for even the harshest of SIP processes.

in this case is much more flexible, and is designed to show overall types of process conditions rather than fixed chemical concentrations. We can also build a qualitative calibration after you've been using it for a while to look back at "good batches" and "bad batches", and give you an indication of where your batch is likely to go over time.

You can run both a **quantitative** and **qualitative** calibration **at the same time** - no need to choose.

### What will I see when I use it?

The IRmadillo software contains its calibration and runs the measurement in real time. This means you'll get an update on chemical concentrations (normally in %wt but that can be changed to whatever units you're used to using) over the whole process.

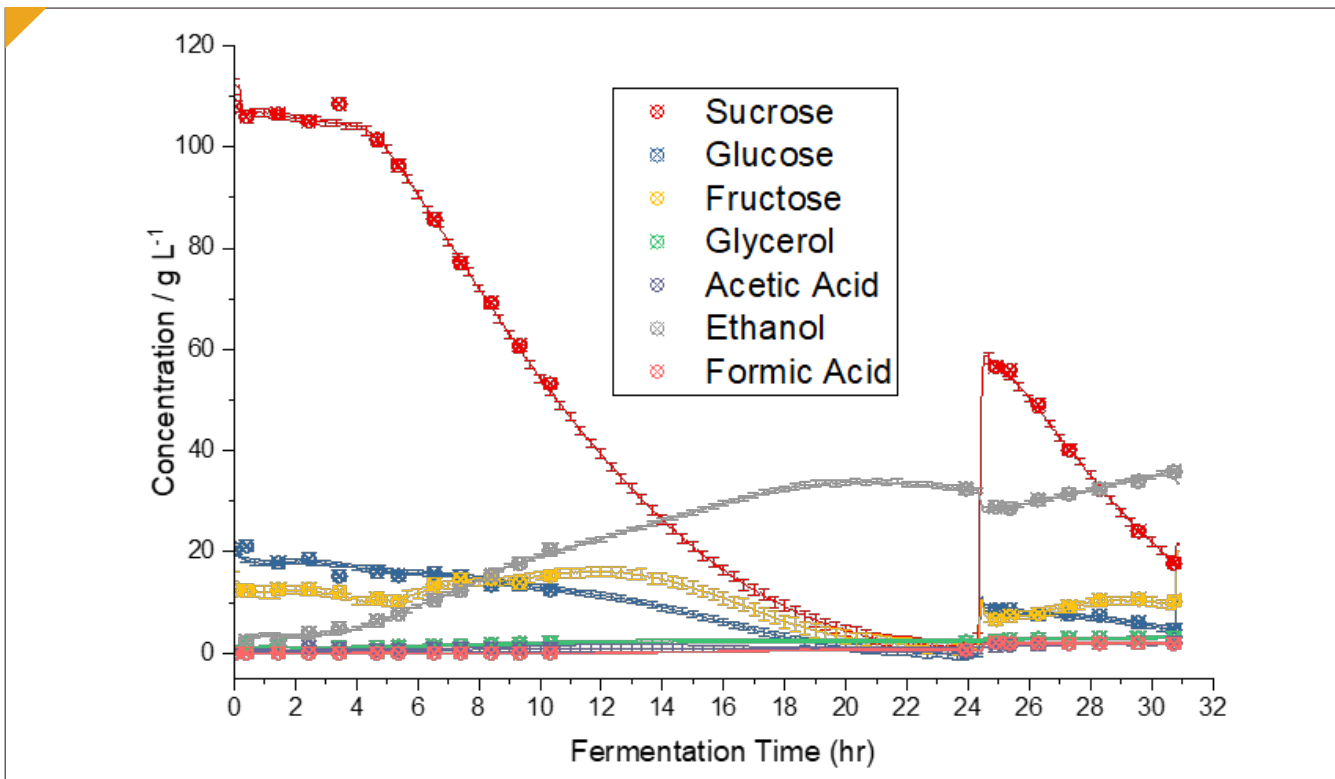
The graph below shows an example sucrose fermentation over 30 hours to give a representation of the output. These batch trends can then be exported into text files for further analysis by your team for process optimisation if needed.

The IRmadillo will also talk to your existing plant communications setup. The standard communications protocols are OPC-UA/Ethernet or Modbus (RTU/RS-485, RS-232 & TCP/Ethernet). Additional protocols can be supported if needed.

### A 38 mm diameter probe is very large – how can I use it with my 1 L fermenter?

The IRmadillo is designed for manufacturing control and monitoring, so can be difficult for laboratory use. If you need to run small-scale (such as 1 L) fermenters to build calibration models we can provide accessories such as flow cells or sample cells to help build the calibration.

If your process is entirely at the 1 L scale you're probably too early in the development pipeline to truly benefit from on-line spectroscopic-based PAT as your process is likely to change too much to build helpful calibrations. But once you're at manufacturing scale we're ready to help!



**Figure 1:** Sucrose fermentation process monitored over 30 hours with the IRmadillo. You can clearly see the change in concentrations of sucrose, glucose, fructose, glycerol, acetic acid, ethanol and formic acid over production time.

## I tried FTIR before and it didn't work...and what about Raman?

Don't confuse **FTIR** (mid-infrared) with **FT-NIR** (near infrared). Infrared light comes in a few different wavelengths, and there was a big push a few years ago to get near infrared instruments (FT-NIR) into plants.

**Near infrared (NIR or FT-NIR)** is very different to the FTIR mid-infrared light that the IRmadillo uses. FT-NIR instruments don't actually look directly at the chemical bonds, but at "overtones". This is a bit like trying to recognise someone from their shadow rather than looking at their face. It gives you a rough idea who it is, but to get full understanding you need the full picture. They also use a probe with a reflecting window in it that can easily get blocked with high solid loadings - such as when there's high biomass in many processes.

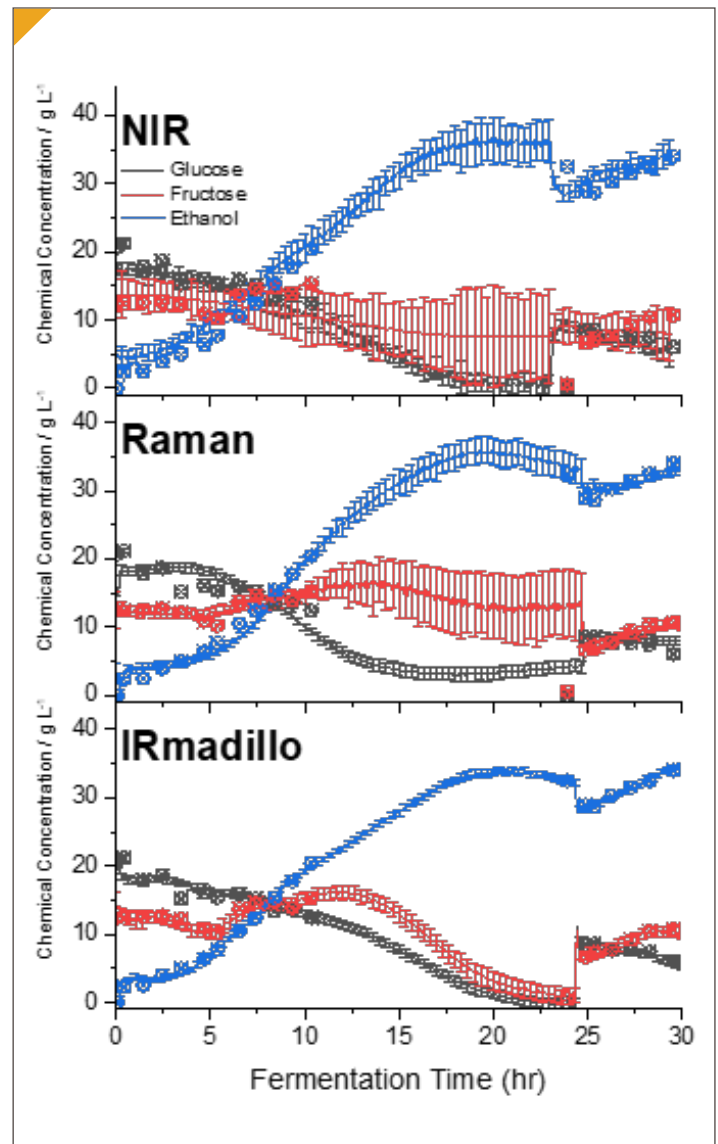
**Raman spectroscopy** looks at similar features to mid-infrared, but the mechanism behind it is completely different. In Raman a laser is used to cause the spectroscopy, and this can be badly affected by fluorescence (which many bioprocesses exhibit) and having biomass or bubbles present. This means in general Raman is not the technique of choice for bioprocesses.

We give an example of this in Figure 2, showing a comparison of an FT-NIR instrument, a Raman and a mid-infrared IRmadillo all monitoring the same sucrose fermentation. The IRmadillo has no trouble monitoring the process and performs well. The NIR simply cannot differentiate between glucose and fructose, making it unreliable and unable to provide any meaningful information. It also shows worse results when the biomass is high - such as before the sugar feed at 23 hr and towards the very end of the process.

The Raman instrument performs better - because it looks at the fundamental information in the same way the IRmadillo does. But it starts to struggle when the biomass is high. This is probably a combination of fluorescence issues and "light scattering" - something that can cause substantial problems with laser based systems such as Raman.

### I have multiple fermenters — do I have to calibrate every instrument?

No! We perform the calibration on one instrument only and can export it across all the IRmadillos you purchase.



**Figure 2:** Comparison of three spectroscopic instruments; near infrared (FT-NIR), Raman and the IRmadillo (FTIR) monitoring a fermentation process over 30 hrs. Note the much larger error bars on the FT-NIR analysis (top) making differentiation nearly impossible between glucose (black lines) and fructose (red lines), while the IRmadillo (bottom) clearly defines each component. The Raman instrument (middle) performs better than the NIR but struggles towards the end of the process and immediately before a sugar feed (at 23 hr). This is due to high biomass at this point - something Raman instruments can have an inherent difficulty with.



## What's the performance of the instrument?

The exact performance depends on the process you use, but a typical error of measurement is shown below.

Chemical	Measurement error / %wt
Sucrose	0.2
Glucose	0.1
Fructose	0.2
Glycerol	0.04
Acetic Acid	0.05
Ethanol	0.7
Formic Acid	0.01

The IRmadillo does not try to out-perform HPLC for error and detection limits, but it can give so much more information over a much shorter time. It also tells you when to take an extract for HPLC — using your staff much more efficiently and effectively.


### Empowering Decisions

The IRmadillo empowers you to understand what your process is doing, and then make informed decisions about how to improve or control it — something that is simply not possible with off-line sampling.

### Interested in finding out more?

Visit our website to read more about our instrument, application notes and technical details.

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

 +44 (0)1235 431260

 [enquiries@keit.co.uk](mailto:enquiries@keit.co.uk)

 [www.keit.co.uk](http://www.keit.co.uk)

## I'm interested. What's next?

Keit gives you options to make it easy to start using an IRmadillo. For all options, we'll help you install, train your team, and we can even calibrate a chemometric model.

### Trial Rental

Want to try one out? Keit will provide an IRmadillo suited to your process operating environment and bill you monthly for an agreed span of time. Easy to renew, you have control over how long you keep it — from a few months to an even longer rent-to-own plan.

### Lease

Consider a longer 2-year leasing plan that is a cost-effective alternative for those needing an instrument, but who also need to keep the CAPEX expenditure in check.

### Purchase

Own your IRmadillo outright to monitor your process on-line and in real time as you see fit.

Begin your discussion today on how you can get an IRmadillo installed into your system.

[enquiries@keit.co.uk](mailto:enquiries@keit.co.uk)

