Application Note

KEIT spectrometers

Conventional FTIR ■ Raman ■ NIR ■ IRmadillo™ FTIR

Comparison of **four different process spectrocopy** instruments in the evaluation of a Bioethanol production process

Abstract

A comparison of four different process spectroscopy instruments was performed on a bioethanol production process; conventional process FTIR, Raman, NIR spectrometers and the novel Keit FTIR spectrometer, the IRmadillo. The Keit IRmadillo has demonstrated its suitability for use in industry to provide real-time online concentration profiles of fermentation processes.

Introduction

The use of process analytical technology (PAT) for bioethanol production is an exciting and potentially very powerful development. Many spectroscopic techniques rely on the vibrational spectroscopies: Fourier transform infrared (FTIR), Raman spectroscopy and near infrared (NIR). Different techniques have their own strengths and weaknesses but are seldom compared to established which is the best technique for a given application.

Keit has developed an innovative FTIR (mid-infrared) spectrometer that contains no moving parts, and removes the need for fragile fibre probes, cooling engines or liquid nitrogen that is required by conventional FTIR instruments.

Here we compare the Keit IRmadillo directly with conventional process FTIR, Raman and NIR spectrometers for in situ monitoring of a bioethanol production process.

Experimental

The fermentation was performed in a 25 L stainless steel Applikon fermenter, with spectrometer probes inserted through standard ports fitted onto the side. The fermentation was performed using *S. cerevisiae* as the organism and sucrose as the feedstock.

IRmadillo™ FTIR Spectrometer

Key Words

- Vibrational spectroscopy
- FTIR
- Raman
- NIR
- Fermentation
- Sugars
- Bioethanol production
- On-line process monitoring

IRmadillo spectra were obtained using 120 s averaging intervals, and the other probes were set to the manufacturer recommendations, with updates every 120 s for direct comparison.

After 24 hr of fermenting time, a second sucrose feed was performed over 15 min to show how the spectrometers performed with a fed batch methodology as well as classical batch processes.

Chemometric models were all built using The Unscrambler 10.5. Various pre-treatments were trialled, but in all cases the extended multiplicative scatter correction (EMSC) was used. Individual PLS-1 models were built for each distinct chemical and instrument combination, so as to avoid any correlation effects, and to allow the models to use the minimum number of factors possible.

Results and Discussion

The root mean squared error for cross validation (RMSECV) is a common technique used to compare performance of models and instruments. The lower the RMSECV, the more precise the model, and – critically – the more trustworthy the predicted value.

Figure 1 shows the RMSECV values for each chemical and each instrument used. (The errors have all been normalised, the maximum value measured for each chemical to avoid artificial bias). It is apparent that for monitoring sugars, (both monosaccharide and disaccharide) and ethanol the IRmadillo exhibits the lowest errors.

The RMSECV is a useful tool for comparing models and instruments, but the real proof lies on comparing the predictions across the entire fermentation, along with offline reference samples.

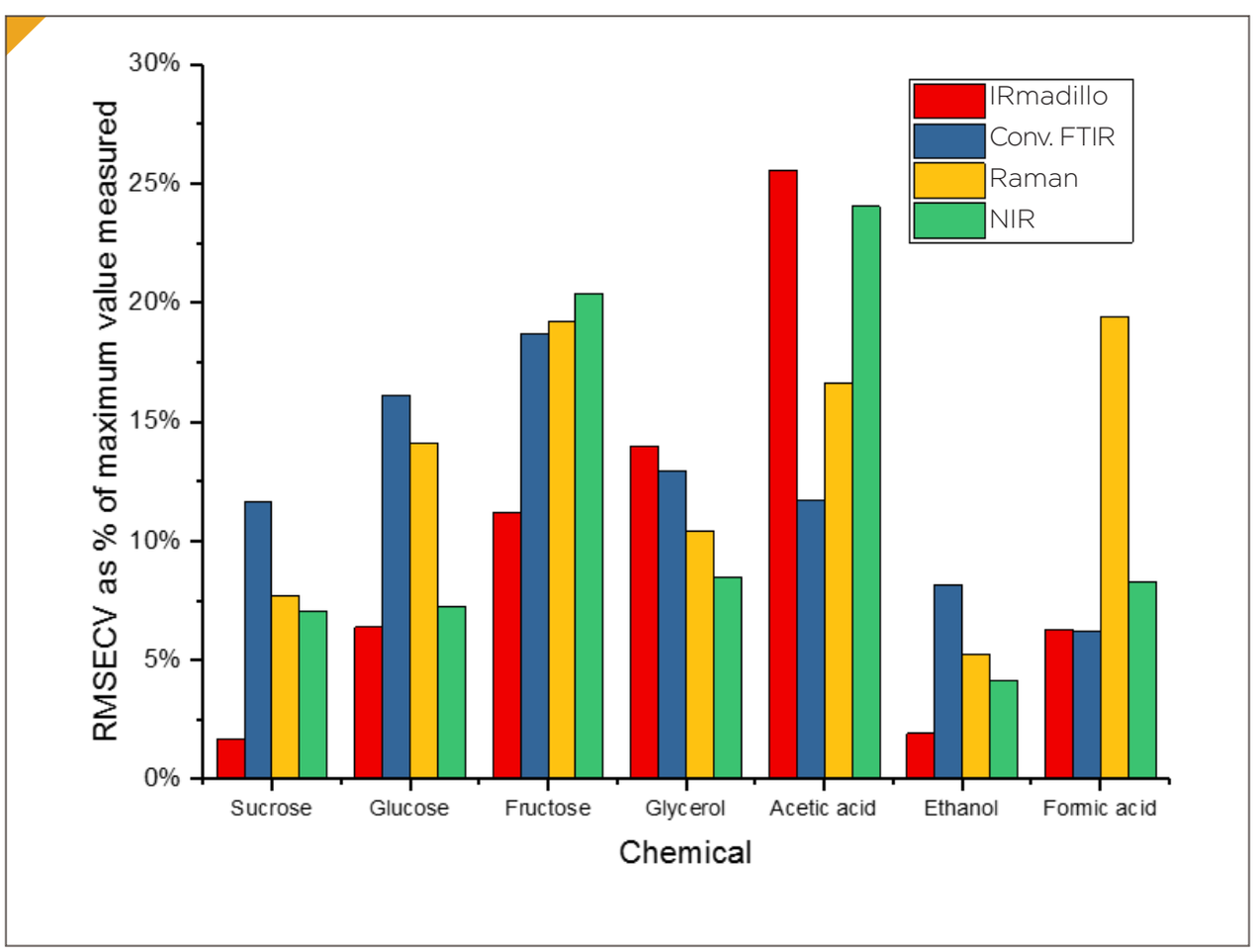

Figure 1: Plot of RMSECV (as a % of the maximum measured value) for each technique and each individual chemical investigated.

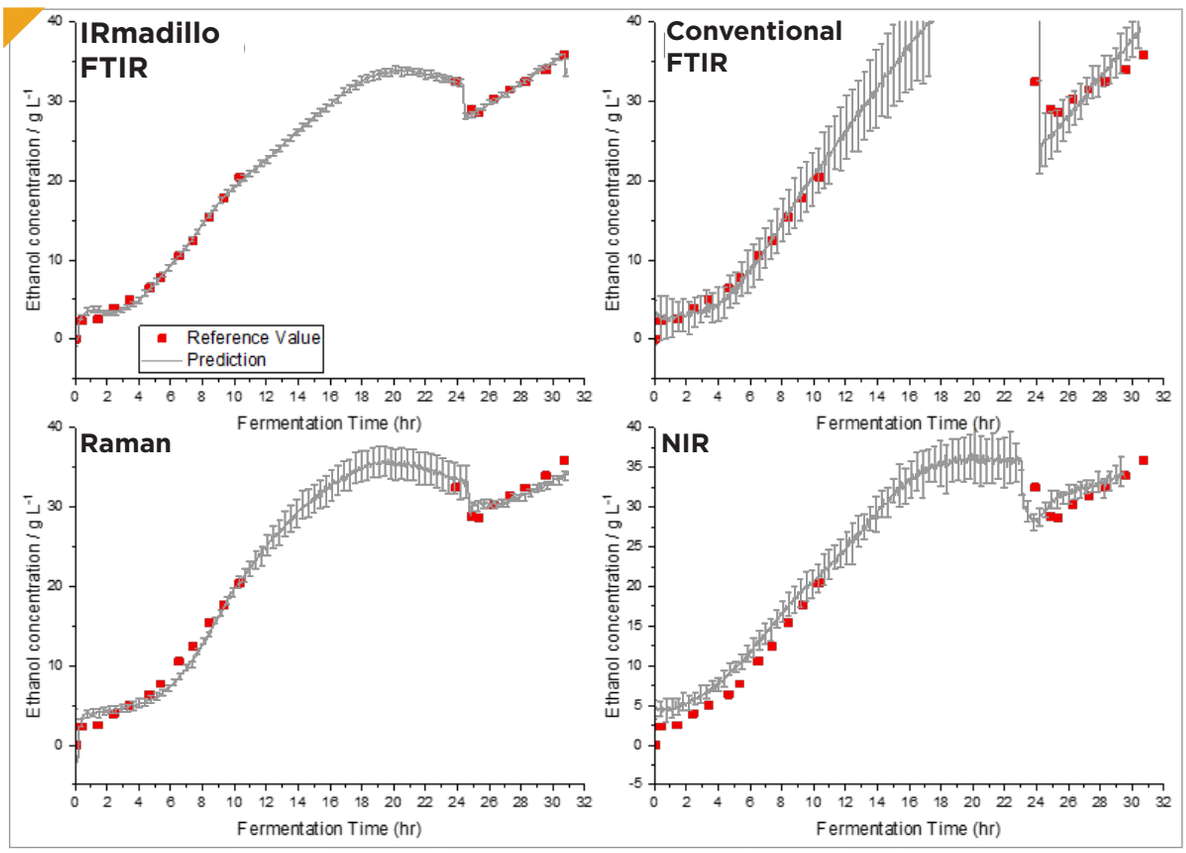

Figure 2: PLS predictions for entire fermentation showing predictions for ethanol concentration.

Figure 2 shows the concentration prediction for ethanol produced over the course of the fermentation. The reference values shown on the graph are derived from off-line HPLC, and are the same in all cases.

IRmadillo FTIR: this shows the best correlation with the reference points, with small error bars and a clear concentration trace the passes through all of the reference points, including those immediately before and after the sucrose feed.

Conventional FTIR: the conventional FTIR can track the initial production of ethanol, but then struggles with the higher concentrations.

Raman: this instrument performs in a similar way to the IRmadillo, but the error bars are larger with greater variation from sample to sample.

NIR: this instrument shows the correct general trend, but is less accurate with a consistent offset on the prediction, over-predicting the ethanol concentration.

Figure 3 shows the concentration prediction for the initial sucrose feedstock, glucose and fructose.

IRmadillo FTIR: this is the only instrument that does not predict negative concentrations for sucrose. The concentration curve for sucrose is clear and correlates extremely well with the reference points. The glucose and fructose measurements are also clearly monitored, including the decrease in all sugar concentrations before the sucrose feed, as well as the rapid increase with feeding.

Conventional FTIR: this does predict the general trend for sucrose, but with a high degree of error and can not accurately measure at low concentrations, showing a negative concentration. The general trend for glucose is measured, but the fructose concentration is not predicted accurately.

Raman: the Raman can not accurately monitor the concentration of sucrose, with notable divergence from the reference values. This is especially noticeable after the feed, where a high biomass concentration is evident. It is likely that fluorescence strongly affected the measurement here. The fructose and glucose concentrations are similar to the IRmadillo predictions.

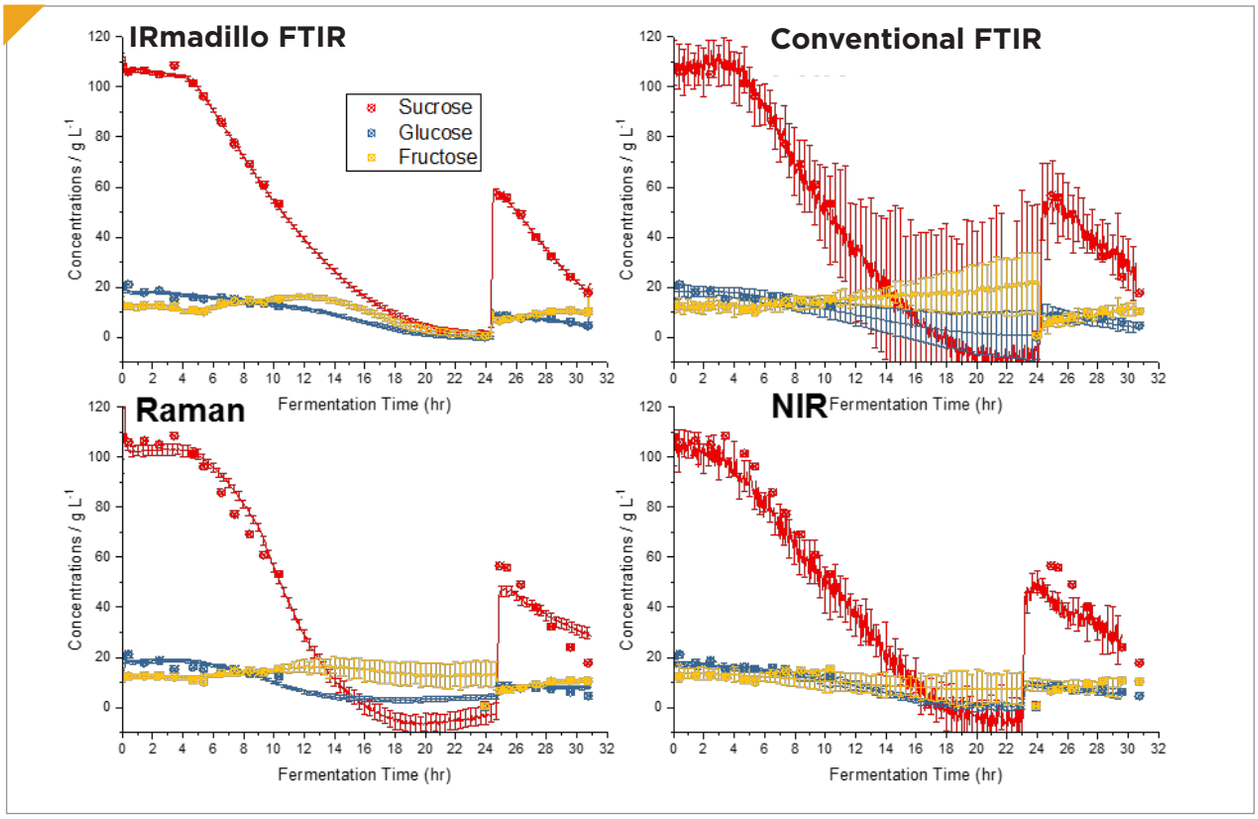

Figure 3: PLS predictions for the entire fermentation showing predictions for sucrose, glucose and fructose consumption and production.

NIR: the NIR shows a reasonable, yet noisy prediction for sucrose in the early stages of the fermentation, but struggles post sucrose feed.

The glucose predictions are fairly accurate, but the NIR cannot monitor fructose prediction at all.

Conclusions

These results shows that the IRmadillo is not only equivalent to alternative conventional process spectroscopies, but is arguably the most accurate technique available.

The improved performance over conventional FTIR comes from the robustness of the solid-state design, meaning no moving mirrors or fibre optics are required. This means that spectral quality is improved, with reliable and stable spectra.

The improvement over Raman comes from the simple design and principals of FTIR compared to Raman, meaning that both fluorescence and scattering effects can safely be ignored.

The improvement over NIR comes from the fact that FTIR spectroscopy directly measures the features of interest, rather than overtones and combination bands. This means similar molecules (such as comparing one molecule of sucrose with one molecule of fructose and glucose) can be identified and quantified much easier than with NIR.

Keep in mind

This application note only shows a few spectra of a much larger project.

If you would like more information on the other molecules measured, please contact us. enquiries@keit.co.uk

IRmadillo™ FTIR Spectrometer

4 Zephyr Building, Eighth Street, Harwell Campus, Didcot, Oxfordshire, OX11 0RL, United Kingdom
+44 (0) 1235 431260 ▪ enquiries@keit.co.uk ▪ www.keit.co.uk