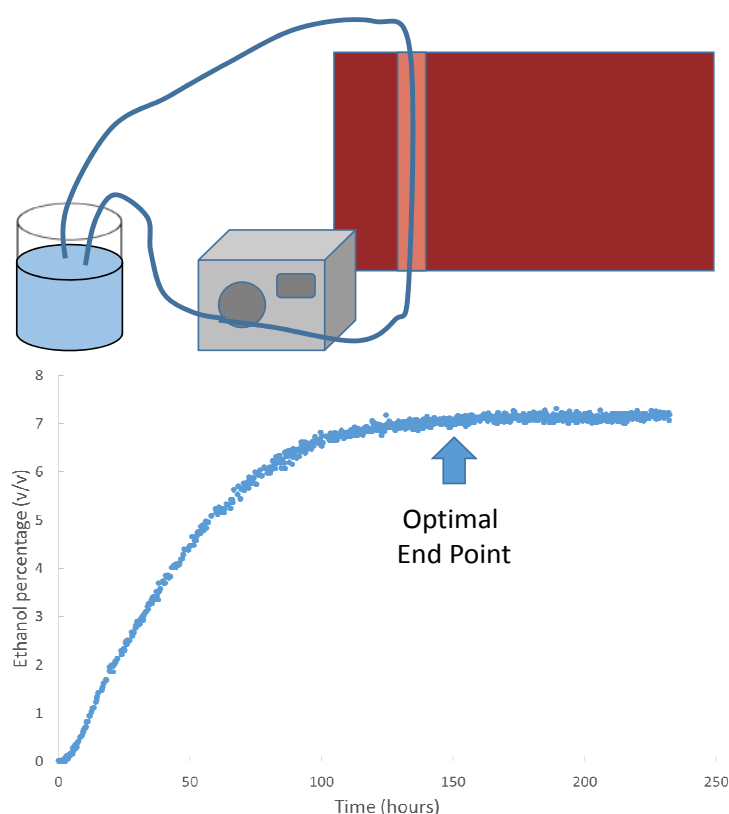


Application Note 6: On-line Reaction Monitoring of Alcoholic Fermentation

NMR spectroscopy is an excellent technique for monitoring organic reactions due to its high degree of functional group specificity. This allows one to integrate over one or more sections of the spectrum to track the increase or decrease of the species of interest.

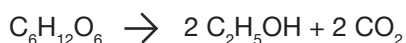
The Spinsolve high-resolution benchtop NMR spectrometer now makes such reaction monitoring extremely easy to set up and run for many reaction processes. It can be installed directly in a fume hood of a chemistry lab to monitor the progress of chemical reactions on-line. Reactants can be pumped in continuous mode from the reactor to the magnet and back using standard PTFE tubing.

Fermentation is a naturally occurring chemical processes – and indeed one of the most popular around the world! In this application note we show how easy it is to monitor a simple fermentation process, and to easily get chemical conversion rates and to see where the optimal end point has occurred.



FERMENTATION

The alcoholic fermentation in juice is governed by the conversion of fructose and glucose into ethanol and carbon dioxide:



The production of carbon dioxide creates the characteristic bubbles observed during fermentation.

A typical ^1H NMR spectrum of a fermenting juice sample is shown in Figure 1. Note that the CH_3 peak of the ethanol is well separated from other peaks in the spectrum. The integral of this peak can be used to quantify the amount of ethanol in the sample.

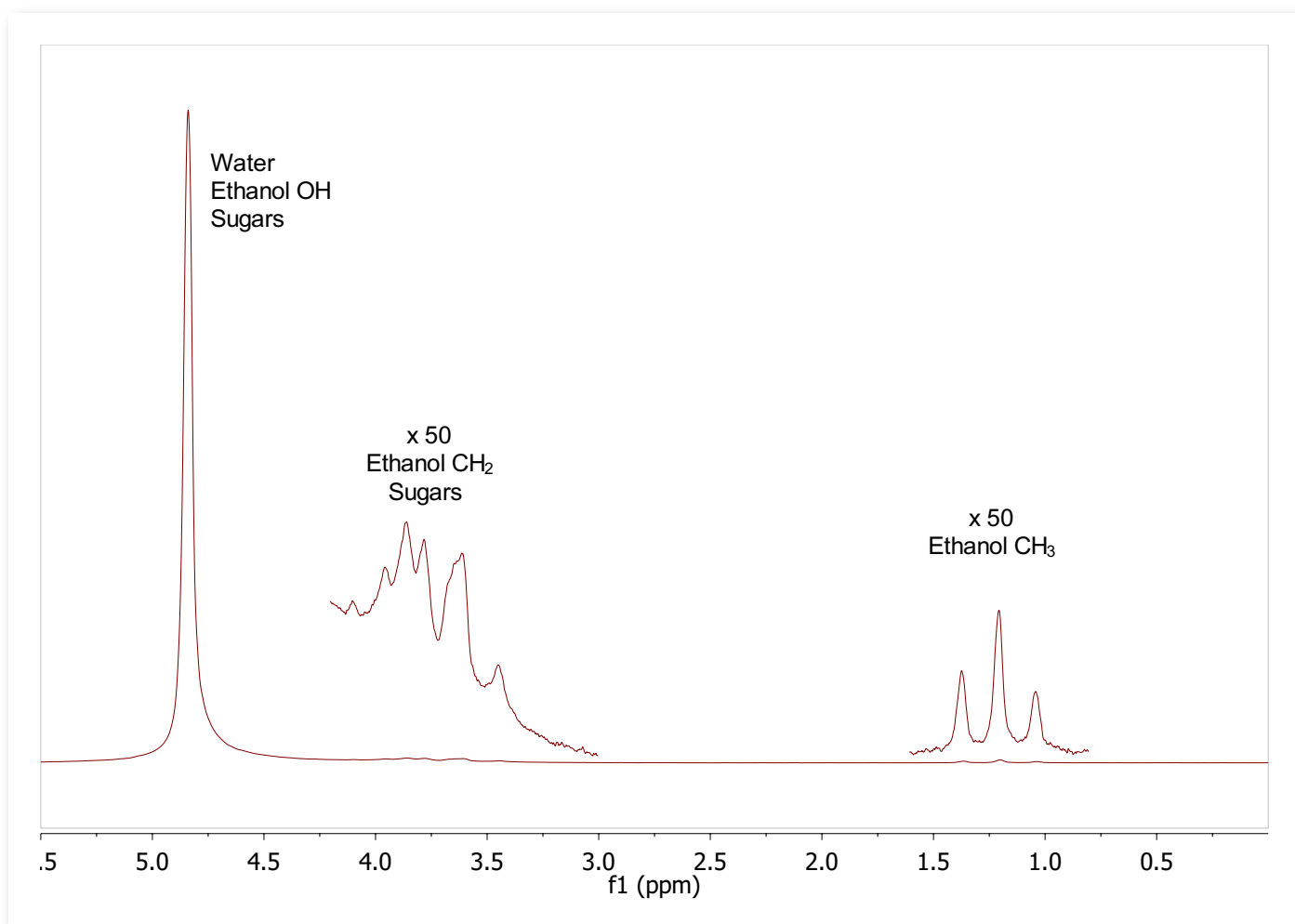


Figure 1: A typical ^1H NMR spectrum of a fermenting juice sample.

FLOW SETUP AND CALIBRATION

A typical setup for on-line monitoring is shown in Figure 2 (a). The reaction mixture is pumped through the bore of the magnet using a 2.5 mm od (1.8 mm id) PTFE tube.

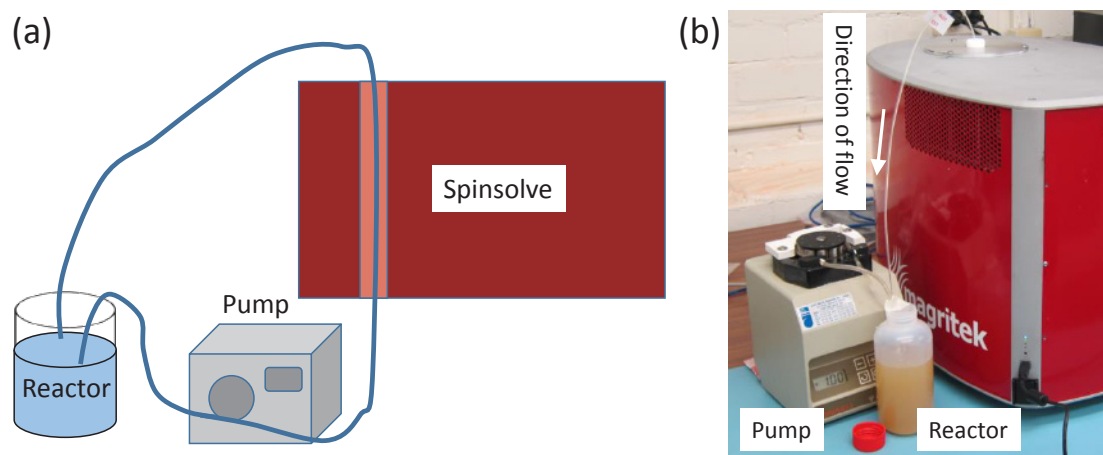


Figure 2: (a) A typical setup for on-line reaction monitoring. (b) Photo of the system setup used to monitor fermentation.

The system was calibrated and shimmed with 400 mL of tap water at a constant flow rate of 0.5 mL min^{-1} . The Reaction Monitoring protocol was then set up to record a 1D Proton spectrum every 30 s. After 5, 20, 35 and 50 minutes, 1 mL of ethanol was injected into the reactor. This corresponds to ethanol volume concentrations of 0.25, 0.5, 0.75 and 1.0%.

Figure 3 (a) shows how the signal of the CH₃ peak changes over time. There is a clear step response in the peak integral about two minutes after each injection. This is the time it takes for the sample to be transferred from the reactor to the measurement volume. It could be shortened by using a transfer tube with a smaller inner diameter, but for the experiment described in this note such a delay was not critical.

The integral graph in Figure 3 (b) provides an easy calibration and also an indication of the accuracy of the measurement. After 60 min, the average integral value is 42.5 units, which corresponds to an ethanol concentration of 1%. The standard deviation of the data is 1.2 units or 0.03% ethanol concentration.

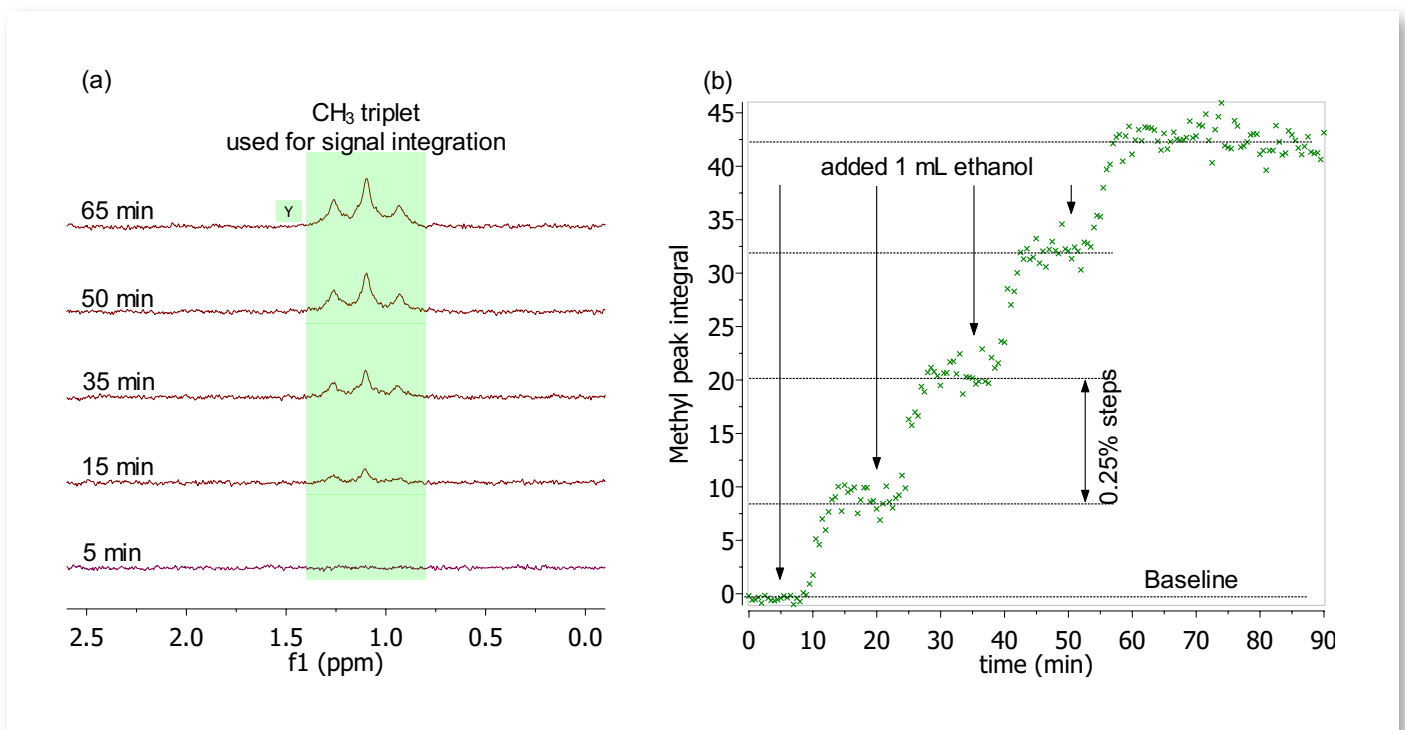


Figure 3: The spectral region of the CH₃ peak over time (a), and the integral of this peak (b)

MONITORING FERMENTATION

For this experiment, 400 mL of apple juice was inoculated with some brewer's yeast. The system was shimmed initially on the flowing juice sample, and the Reaction Monitoring protocol was then set up to record a 1D Proton spectrum once every 10 min. A photo of the setup is shown in Figure 2 (b).

Figure 4 shows some spectra taken during the first hours of the reaction (a) and the methyl peak integral (b). Since the fermentation produces CO_2 gas, it is inevitable that gas bubbles will eventually reach the measurement volume. As a result, the spectrum will be severely distorted if a gas bubble is present during an acquisition. A good example can be seen at 10 hours in Figure 4 (a). The integral values of such bad spectra can easily be identified as outliers in Figure 4 (b) and filtered out when processing the data.

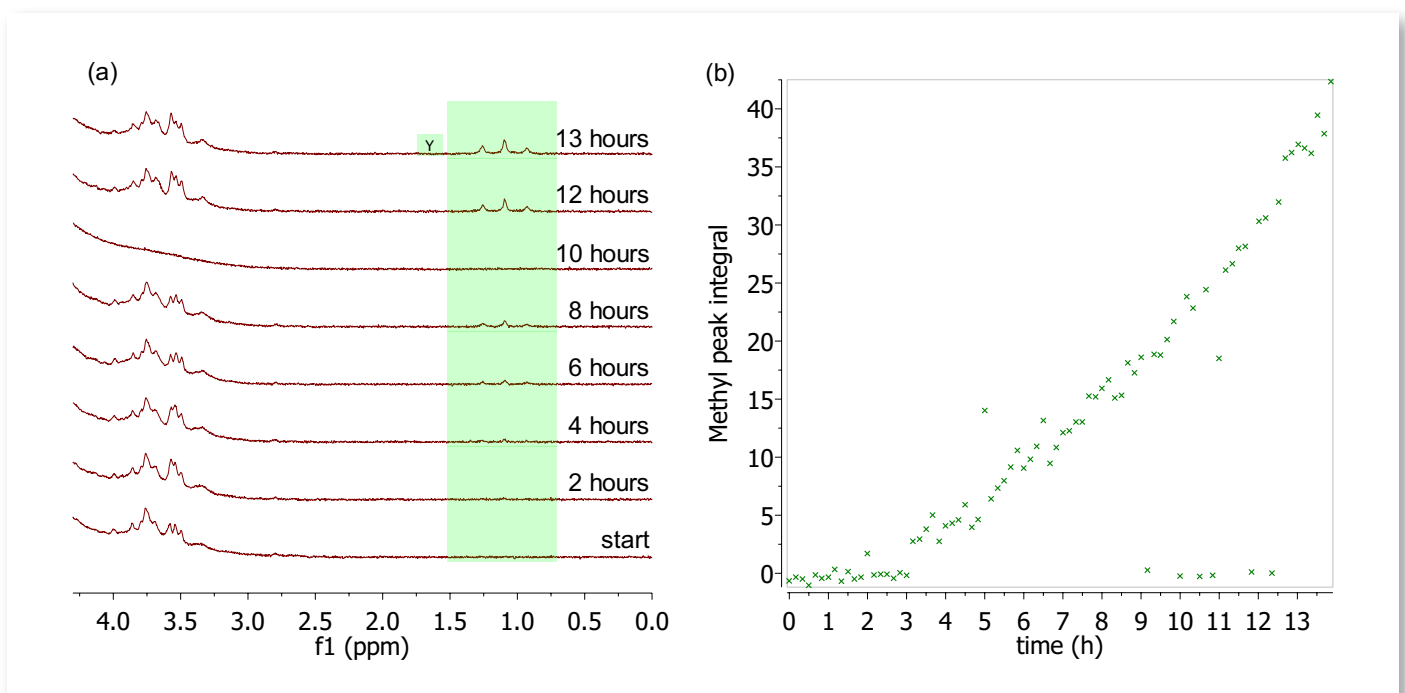


Figure 4: The first 13 hours of the fermentation. (a) Spectra, (b) methyl peak integral

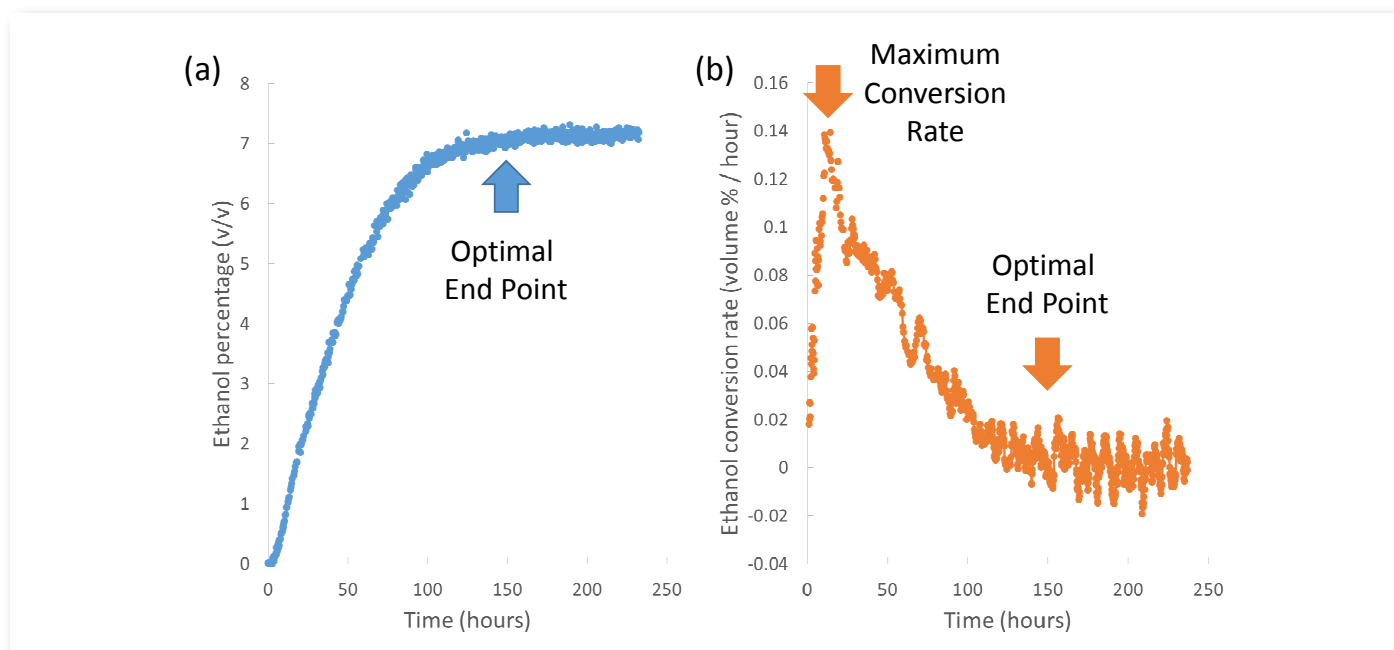


Figure 5: (a) The ethanol percentage as measured on-line during a fermentation process after filtering distorted data points. The slope of this curve gives the ethanol conversion rate (b).

The fermentation process was monitored for a total duration of ten days. With the calibration data from Figure 3 (b) the ethanol percentage and conversion rate can be quantified. This is shown in Figure 5. The start and end points of the reaction are easily identified after 3 and 150 hours, where the conversion rate is basically zero. The conversion rate reaches a maximum at 14 hours after inoculation.

This shows that on-line reaction monitoring is a valuable tool, as it enables the user to take appropriate action if the process goes along an unexpected path or when the reaction reaches its end point.

Conclusions

It has been shown that a simple flow loop setup allows on-line reaction monitoring on a lab bench. Shimming was performed at the start of the experiment on the reactant itself. No extra shimming was required during the ten day long experiment, although it is possible if necessary. Even gas bubbles in the measurement volume did not disrupt subsequent measurements.

CONTACT INFORMATION

For further information, please contact: sales@magritek.com

GERMANY

Philipsstraße 8
52068 Aachen, Germany
Tel: +49 (241) 70525-6000
Fax: +49 (241) 963 1429

NEW ZEALAND

6 Hurring Place, Unit 3
Newlands, Wellington 6037, NZ
Tel: +64 4 477 7096
Fax: +64 4 471 4665

UNITED STATES

6440 Lusk Blvd (D108)
San Diego, CA 92121, USA
Tel: +1 (855) 667-6835
+1 (866) NMR-MTEK

Or visit our website www.magritek.com