

Monitoring Bioethanol Production with the Thermo Scientific Prima PRO and Prima BT Process Mass Spectrometers

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Key Words

- Fermentation
- Lignocellulosic Biomass
- Cellulosic Ethanol
- Thermophiles
- Magnetic Sector
- Rapid Multistream Sampler



Introduction

Bioethanol is the term used to describe ethanol derived from a biochemical process rather than a chemical process. The majority of the world's bioethanol is produced by the fermentation of sugars derived from sugar cane and starch from corn and wheat using yeast – a process very similar to traditional brewing. However, there are growing concerns that using food crops to produce transport fuels will lead to competition for these feedstocks and rising food prices. A new generation of bioethanol processes are therefore being developed that use low value, non-food based feedstocks known as lignocellulosic biomass. This includes agricultural residues (e.g. corn stover and sugarcane bagasse), wood residues (e.g. discards from sawmills and paper mills) and municipal paper waste. The product is called cellulosic ethanol and represents the next generation of sustainable green fuel, helping to reduce greenhouse gas emissions and dependence on crude oil.

Ethanol is already an accepted transportation fuel in many countries. Most spark-ignited gasoline engines will operate without modification with anhydrous ethanol blended into the gasoline at levels up to 10%, called E10 gasoline. In 2013, almost 30 billion US gallons of ethanol were produced worldwide, compared to 17 billion US gallons in 2007 (Renewable Fuels Association).

Bioethanol Production

Bioethanol is produced in one of two ways. One approach is to burn the biomass to generate synthesis gas (carbon monoxide, hydrogen and carbon dioxide) then ferment the synthesis gas (also known as syn gas) to ethanol. The other approach avoids the need for a combustion stage by breaking down the biomass prior to fermentation. There are three main production processes available for this route:

1. Separate Hydrolysis and Fermentation (SHF)

Enzymes are used to hydrolyse the cellulose materials to sugars which are then fermented to ethanol.

2. Simultaneous Saccharification and Fermentation (SSF)

Saccharification, the process of converting complex carbohydrates into sugars, and fermentation of the sugars into ethanol, take place in the same reactor.

3. Consolidated Bioprocessing (CBP)

Consolidated bioprocessing combines four biological processes (production of saccharolytic enzymes, hydrolysis of the carbohydrates in biomass, fermentation of hexose and pentose sugars) in one reactor.

Whichever method is chosen to break down the biomass, there is a need to ferment sugars to produce bioethanol. The fermentation can either use yeast to convert the carbohydrates to carbon dioxide and ethanol, as in conventional brewing processes. Alternatively high temperature-loving microorganisms called thermophiles can be used at temperatures in excess of 60°C. Thermophiles can give better yields, are more robust and utilize a wider range of biomass feed-stocks such as agricultural waste and green refuse. Unlike yeast, thermophilic bacteria can ferment the pentose sugars derived from hydrolysis of these waste products. They can also be used in a continuous process which is more efficient than the traditional batch type fermentation process.



Figure 1: Typical small-scale benchtop fermentor for bioethanol production.

Analytical Requirements

A typical small-scale benchtop fermentor for bioethanol production research is shown in **Figure 1**. As in any conventional fermentation it is important to monitor the metabolic state of the micro-organisms according to the oxygen they consume and the carbon dioxide they produce. In the production of bioethanol it is also important to monitor the concentration of ethanol in the vent gas to determine the fermentor's ethanol production rate. The ethanol concentration in the broth is typically measured using liquid chromatography but this is an off-line measurement taken only periodically throughout the fermentation. While it is useful as a reference it does not give a continuous measurement, hence no information on process kinetics.

Advantages of Gas Analysis Mass Spectrometry

Fermentation scientists in a wide range of biotechnology industries have been using Thermo Scientific™ process mass spectrometers since the early 1980s. They monitor the composition of gas streams into and out of fermentors and bioreactors continuously, accurately and reliably. Unlike discrete analyzers, they monitor all the air gases – oxygen, carbon dioxide, nitrogen and argon. They have also been used to monitor a wide variety of volatile organics including ethanol. Since the concentration of ethanol in the vent gas is linearly related to the concentration in the fermentor broth, they give a continuous monitor of the ethanol production which is particularly important for detecting the start of ethanol production and also for monitoring changes in ethanol production.

Advantages of Thermo Scientific Prima Gas Analysis Mass Spectrometers

The manufacturing process typically begins with cell cultures grown in the laboratory. Then, during the scale-up process, cells are sequentially transferred to larger and larger fermentors, eventually into production vessels that can hold up to 20,000 litres of growth media and cells.

It is vital to maintain the precise environment that specific cells need to remain healthy and grow – this requires precise off-gas analytical data through every stage of the scale up process, from laboratory to pilot plant to bulk production. In some cases one mass spectrometer (MS) fitted with a suitable RMS multi-stream inlet can monitor all the fermentors, while in other cases separate MS analyzers have to be used in the laboratory and in the plant. It is critical that results from the two analyzer platforms correlate to ensure a smooth transition through the various stages of scale up.

Figure 2 shows an example of a mass spectrometer suitable for fermentation process development, the Thermo Scientific Prima BT benchtop MS. **Figure 3** shows an example of a mass spectrometer suitable for production process monitoring, the Thermo Scientific Prima PRO process MS. Both systems use the Thermo Scientific magnetic sector analyzer, which has key advantages over alternative quadrupole analyzers including improved precision, accuracy, long intervals between calibrations and resistance to contamination. Typically, analytical precision is between 2 and 10 times better than a quadrupole analyzer, depending on the gases analyzed and complexity of the mixture.

We manufacture both quadrupole and magnetic sector mass spectrometers; over thirty years of industrial experience have shown the magnetic sector based analyzer offers the best performance for industrial online gas analysis.

A unique feature of the Prima magnet is that it is laminated. Our analyzer scans at speeds equivalent to that of quadrupole analyzers, offering the unique combination of rapid analysis and high stability. This allows the rapid and extremely stable analysis of an unlimited number of user-defined gases. The scanning magnetic sector is controlled with 24-bit precision using a magnetic flux measuring device for extremely stable mass alignment.

Component	Concentration Range (% mol)	Standard Deviation*
Nitrogen	0-100	0.005 %mol
Oxygen	0-100	0.005 %mol
Argon	0-1	0.001 %mol
Carbon dioxide	0-10	0.1% relative or 0.0003 %mol**
Methanol	0-1	2% relative or 0.001 %mol**
Ethanol	0-1	2% relative or 0.001 %mol**

*Whichever is greater.

Figure 4: Typical Prima PRO Process Mass Spectrometer performance specification for fermentation. Analysis time, including switching time, is 20 seconds per stream for the six components.



Figure 2: Thermo Scientific Prima BT process development mass spectrometer.



Figure 3: Thermo Scientific Prima PRO process mass spectrometer.

A typical fermentation performance specification is shown in **Figure 4**.

The Prima PRO and Prima BT mass spectrometers meet the single standard deviations quoted based on an analysis time of 20 seconds, including stream switching for the six species listed.

Thermo Scientific GasWorks software permits analysis optimization on a per-stream basis so the most appropriate speed versus precision setting can be selected, depending on process control requirements. Similarly, the most efficient peak measurements for each stream and the most appropriate display units (% or ppm) can also be selected.

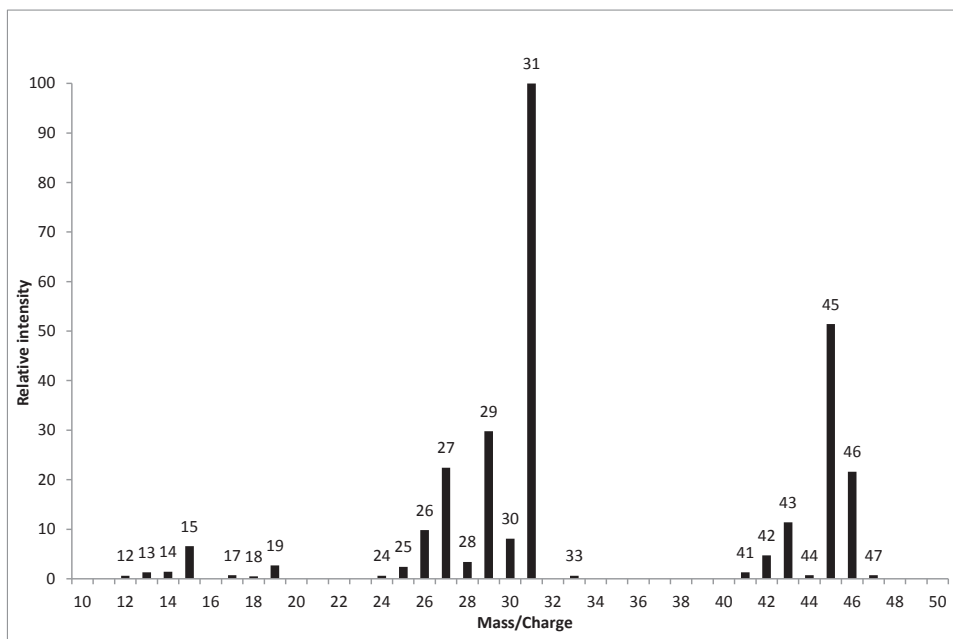


Figure 5: Ethanol fragmentation pattern.

we need to consider the presence of a very large peak at mass 32 from the percentage levels of O_2 in the vent gas. We need to correct for the tail from peak 32 to make an accurate measurement of ethanol at low concentrations (ppm) at the start of ethanol production. This is vitally important to the whole fermentation process. The intensity of the tail from O_2 at mass 31 compared with the intensity of the peak at mass 32 is 0.02%. When the concentration of O_2 is around 20%, this means the signal at mass 31 is equivalent to around 40 ppm. During calibration this interference is recorded so that subsequent analysis is corrected accordingly. On a quadrupole instrument this interference level is much greater and also variable, resulting in excessive uncertainty for low level ethanol measurement. A low level ethanol signal effectively tends to get 'buried' in the noise from the oxygen peak. With the Prima PRO magnetic sector instruments the measurement is very reproducible and ethanol can be measured with a precision down to 10 ppm. **Figure 6A** shows the mass spectrum around mass 31 for air with no ethanol present, **Figure 6B** shows the logarithmic spectrum for air containing approximately 400 ppm ethanol.

Ethanol Calibration

The MS is calibrated for air gases using calibration cylinders, but due to the low vapour pressure of ethanol it is not possible to buy compressed gas cylinders containing ethanol at high concentrations. Even if the ethanol concentration is just 0.04%, the maximum cylinder pressure that can be supplied is about 10 bar. There can also be issues with ethanol adsorption in the cylinders, leading to cylinders with nominally identical concentrations giving different readings. To obtain the best possible ethanol calibration we therefore recommend a vaporization device.

A vial containing ethanol liquid is placed in a temperature controlled oven chamber. The vial is designed with a capillary of appropriate length and diameter to provide the required rate of ethanol evaporation through diffusion (Valco Instruments Company Inc). A known flow of carrier gas flows through the oven chamber. By measuring the weight loss of the vial containing ethanol over a period of time (e.g. 8 hours), the ethanol evaporation rate (in units of volume of vapour per unit time) can be calculated and hence the concentration of ethanol in the carrier gas can be accurately determined.

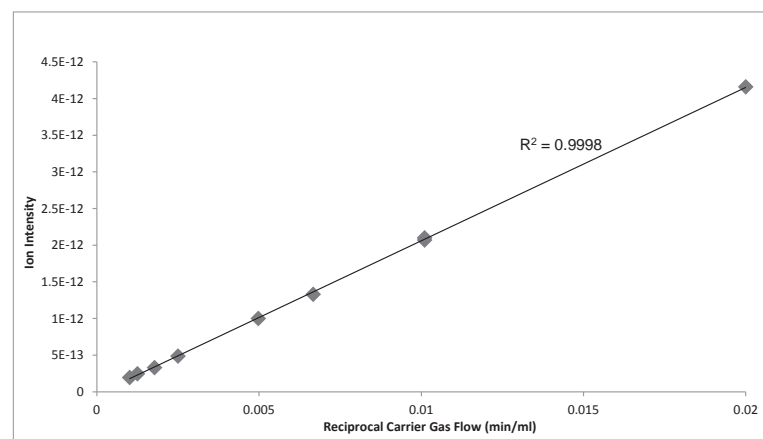


Figure 7: Ethanol calibration linearity from 230 ppm to 4600 ppm.

Ethanol Analysis

The accurate online analysis of ethanol is essential to understand the fermentation process kinetics and to close the mass balance. **Figure 5** shows the mass spectral fragmentation pattern for ethanol. Although the molecular weight of ethanol is 46 it can be seen that the molecular ion ($CH_3CH_2OH^+$) peak at mass 46 is actually not the largest peak, in fact it is not even the second largest peak. The ethanol molecule tends to fragment during ionization and the largest peak is actually at mass 31 due to CH_3O^+ . Also there is considerable interference from the CO_2 in the vent gas at masses 45 and 46, due to the ^{13}C , ^{17}O and ^{18}O isotopes. Therefore, we have to use mass 31 to analyze ethanol. However,

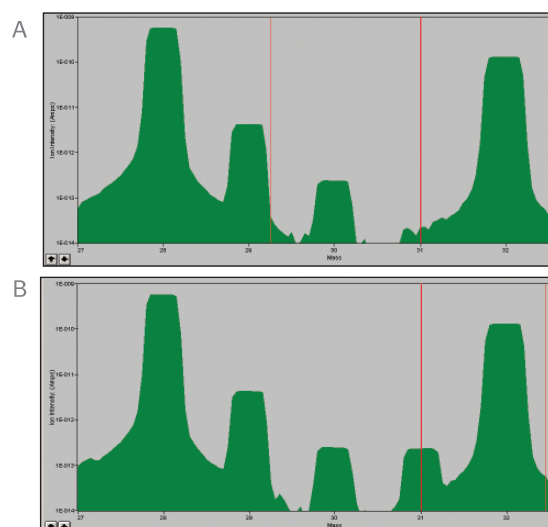


Figure 6: A. Spectrum of air without ethanol. B. Spectrum of air with 400 ppm ethanol.

To check on instrument linearity, the concentration of ethanol can be varied by adjusting the carrier gas flow rate. The concentration of ethanol is simply inversely proportional to carrier gas flow rate.

Figure 7 shows an example of the linearity of the measurement, tested over a flow range of 1000 ml/min down to 50 ml/min (giving a concentration range of 230 ppm up to 4600 ppm).

The measurement is stable within the limits of the temperature

stability of the oven. It should be noted that a $\pm 0.2^{\circ}\text{C}$ variation in temperature at 55°C causes approximately $\pm 2\%$ relative change in ethanol concentration.

Example data from repeated relative (to N_2) sensitivity calibrations over 15 days are shown in **Figure 8**. The relative standard deviation of the repeated ethanol relative sensitivity determination was around 4% relative. We believe the variation observed is due to weighing errors and flow measurement errors. However, the stability observed is still considerably better than the stability observed using calibration cylinders. Uncertainty in calibration is reduced by performing the calibration several times over a period of time and using an average value, rather than a single point value. The actual ethanol relative sensitivity of the instrument is very stable and, once calibrated, should not need updating more often than 6 monthly or whenever any maintenance is performed on the instrument (either scheduled or unscheduled).

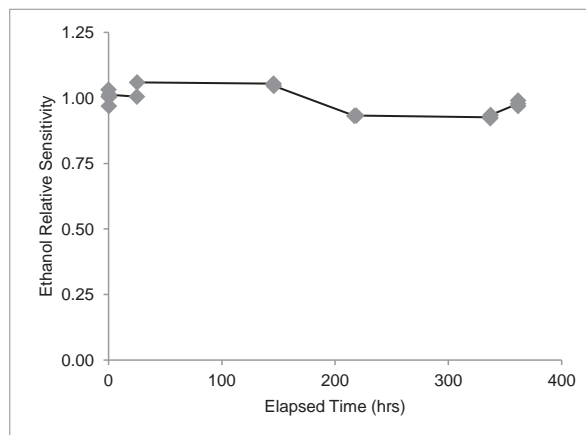


Figure 8: Ethanol sensitivity over 15 days.

Results

An example of an online analysis trend for ethanol in vent gas for a short batch experimental run is shown in **Figure 9**. The sudden fall off at 16:00 was caused by a problem with the air intake causing a drop off in the aeration rate. This was immediately indicated by the MS and subsequently rectified. Without the online data from the MS the culture would have been starved of oxygen, causing a catastrophic reduction in ethanol yield. Now the process operates normally and the ethanol vapor is measured at a steady target concentration.

Summary

The Thermo Scientific Prima family of gas analysis mass spectrometers offer the best available online measurement precision and stability for fermentation process monitoring and control, whether it is the Prima BT MS in the development laboratory or the Prima PRO MS in the production plant. They have been used in fermentations producing cellulosic bioethanol, other biofuels such as biobutanol, and a wide range of biopharmaceutical and biotechnology products. Fault tolerant designs combined with extended intervals between maintenance and simplified maintenance procedures ensure maximum availability.

- Online measurement of alcohols, O_2 , CO_2 , N_2 , Ar, CER, OUR, RQ etc.
- Linear, reproducible measurement of alcohols
- Long periods between calibration

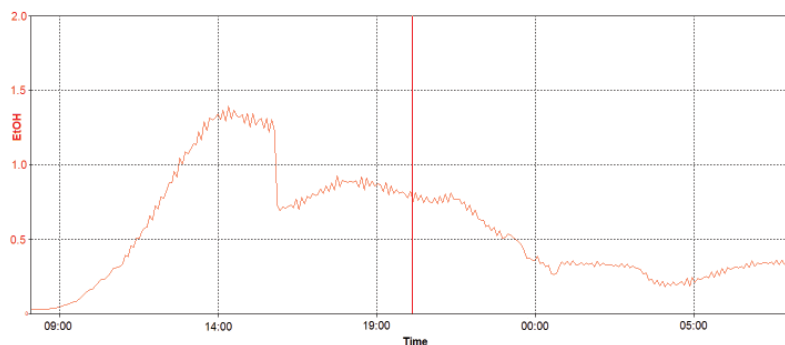


Figure 9: Trend display of ethanol from batch fermentation.

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