

The Application of Process Mass Spectrometry to Bioethanol Production

Key Words

- Prima δ B
- Bioethanol
- Cellulosic Ethanol
- Fermentation
- Mass Spectrometry
- Online Analysis

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 or the fermentation of starch derived from corn and wheat using yeast, a process very similar to traditional brewing. Increasingly, however, it is recognized that using food crops to produce transport fuels may lead to competition for these feedstocks, thus causing an increase in food prices.

A new generation of bioethanol processes is being developed that uses low-value, non-food feedstocks, such as waste agricultural residues, wood waste, straw, bagasse and corn stover. Ethanol produced from these biomass materials is referred to as cellulosic ethanol. It represents the next generation of sustainable green fuel which will help reduce greenhouse gas emissions and dependence on external oil imports and, in turn, reduce concerns about energy security.

Ethanol is already an accepted transportation fuel in many countries, including Brazil and the U.S. Most spark-ignited petroleum (gasoline) engines will operate well without modification using ethanol-blended gasoline, when up to 10% is ethanol (i.e., E10). In 2007, total global production was 13 billion U.S. gallons, with 6.5 billion produced in the U.S. and 5 billion produced in Brazil. Statistics on ethanol production are available on the Renewable Fuels Association web site at www.ethanolrfa.org.

The largest expense associated with ethanol production is the cost of the feedstock, such as corn, grain, etc. These costs could be reduced significantly if inexpensive waste plant biomass-based materials (i.e., straw, corn stover and bagasse) could be used. One approach is to generate

synthesis gas, which is comprised of carbon monoxide, hydrogen and carbon dioxide, by burning the biomass, then fermenting this synthesis gas to ethanol. U.K.-based TMO Renewables is taking a different approach. The company is developing a novel second-generation cellulosic ethanol technology that can quickly and efficiently convert these biomass wastes into ethanol. This technology is based on the use of high temperature-loving microorganisms, called thermophiles, which are able to convert the wide diversity of simple and complex sugars found in biomass into ethanol at temperatures in excess of +60°C. This application note discusses the use of the Thermo Scientific Prima δ B mass spectrometer for monitoring the process.

Using Thermophiles to Optimize Ethanol Yields

TMO Renewables is evaluating different strains of microorganisms under varying fermentation conditions to optimize the ethanol yield. This technology is different from conventional ethanol production (such as in brewing) in that, rather than using yeast, special thermophiles are used which give better yields, are more robust and can utilize a much wider range of biomass feedstocks, including any sort of agricultural waste or green refuse. Also, a continuous process is being developed which is much more efficient than the traditional batch-type fermentation process. Unlike yeast, TMO Renewables' thermophilic bacteria are able to utilize the pentose sugars derived from the hydrolysis of agricultural waste.

Currently, TMO Renewables is operating at lab/pilot scale in their research laboratories in Guildford, England and has just completed construction of the U.K.'s first cellulosic ethanol demonstration facility which is located nearby. The company is using the Prima δ B in



Thermo Scientific Prima δ B

both the lab and the demonstration facility to monitor the concentration of ethanol in the vent gas as well as the metabolic state of the microorganisms according to the O₂ consumed and the CO₂ evolved. The online analysis of ethanol concentration in the vent gas allows determination of the ethanol production rate by the fermenter, with the concentration of ethanol in the vent gas related linearly to the concentration in the fermenter broth. The ethanol concentration in the broth is typically measured off-line using liquid chromatography, periodically throughout the fermentation, as a reference. However, online mass spectrometry via the Prima δ B is particularly useful because it enables continuous monitoring of the ethanol production which is important for detecting the start of ethanol production as well as changes during production. The accurate online analysis of ethanol is essential to understand the process kinetics and to close the mass balance. The Prima δ B is therefore a cornerstone in this analytical

functionality and provides invaluable data not only on ethanol evolution but also on O₂ consumption and CO₂ production, providing critical data on the state of the fermentation and facilitating optimization efforts.

Analysis of Bioethanol Production Vent Gas

The analysis of the vent gas was configured per the chart in *figure 1*, indicating the particular ions that were selected for monitoring. The analysis was on a normalized dry basis (i.e., the individual reported component concentrations were adjusted with their sum, excluding water, to equal 100%). The concentrations were calculated based on peak heights. The peak height response was linear to within 1% relative over a decade change in concentration and was calibrated using calibration gases (*figure 2*).

The biggest challenge in analyzing the vent gas was obtaining reliable calibration and analysis for ethanol. The Prima δB was essential to the process because it offers magnetic sector mass spectrometry to establish reliable calibration, rather than a quadrupole-type analyzer which does not provide sufficient resolution to allow precise monitoring of the ethanol peak. In the ethanol spectrum, *figure 3* shows the main peaks observed using the Prima δB.

Figure 3 indicates the molecular ion (CH₃CH₂OH⁺) peak at mass 46 was not actually the largest peak. In fact, it was not even the second largest peak. Ethanol has a tendency to fragment during ionization, and the largest peak was actually at mass 31 due to CH₂O⁺. Also, there was considerable “interference” from CO₂ in the vent gas at masses 45 and 46 due to the C-13, O-17 and O-18 isotopes. Therefore, mass 31 was used for analysis. However, consideration had to be given to the proximity of the very large peak due to O₂ at mass 32. A tail was present which had to be corrected for in order to make an accurate measurement of ethanol at low concentrations (ppm) at a critical point: the start of ethanol production. The intensity of the tail from O₂ at mass 31 compared with the intensity of the peak at mass 32 was 0.02%. When the concentration of O₂ was ~20%, the signal at mass 31 was equivalent to ~40 ppm. During calibration, this interference was recorded and

	Measured Range of Concentrations	Molecular Ion and Fragment Ion peaks used for analysis (expressed relative to base peak)	Comments
N ₂	Balance	28 = 100	
O ₂	10-21% vol	32 = 100 and 31 = 0.02	31 is a low level tail used to correct the ethanol peak height (see below)
Ar	0.8-1.0% vol	40 = 100	
CO ₂	0-10% vol	44 = 100 and 28 = 5.5	
Ethanol	0-10% vol	31 = 100	Note: the fragment ion peak at mass 31 is used instead of the molecular ion peak at mass 46 because it is more sensitive (see below)

figure 1 – The selected ions used for analysis of the vent gas.

Calibration Gases	Calibration Gas 1	Calibration Gas 2	Calibration Gas 3	Calibration Gas 4	Calibration Gas 5
	Cylinder mixture	Cylinder pure gas	Cylinder mixture	Vapor generator	Instrument air
N ₂	Balance		Balance	Balance	78.08
O ₂	15% vol				20.95
Ar	1% vol				0.934
CO ₂	5% vol		5% vol		0.036
Ethanol				0.3% vol	
Helium		100% vol			
Comment	Used for calibrating response of N ₂ , O ₂ , Ar and CO ₂	Used for calibrating for background readings at masses 28, 31, 32, 40 and 44	Used for calibrating fragmentation pattern of CO ₂	Used for calibrating response of ethanol	Used for calibrating fragmentation pattern of CO ₂

figure 2 – The peak heights are calibrated by the Prima δB using calibration gases.

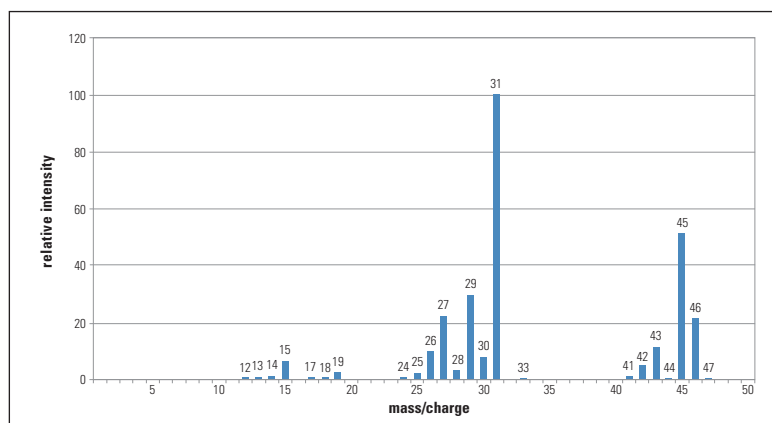


figure 3 – Ethanol relative intensity by mass/charge.

subsequent analysis was corrected accordingly. On a quadrupole instrument, however, this level is much greater and also variable, resulting in excessive uncertainty in low level ethanol measurement. In addition, a low level ethanol signal tends to get effectively “buried” in the noise. Using the Prima δB’s magnetic sector technology, the measurement is very reproducible and ethanol can be measured with a precision down to ~10 ppm. *Figure 4* shows the spectrum around

mass 31 for air without any ethanol present and *figure 5* represents the (logarithmic) spectrum for air containing ~400 ppm ethanol. Another issue is obtaining a reliable calibration gas. Due to the low vapor pressure of ethanol, it is impossible to buy compressed gas cylinders containing ethanol at high concentrations. Even at 0.04% ethanol, the maximum cylinder pressure that can be supplied is close to 10 bar. In addition, different cylinders containing the same amount

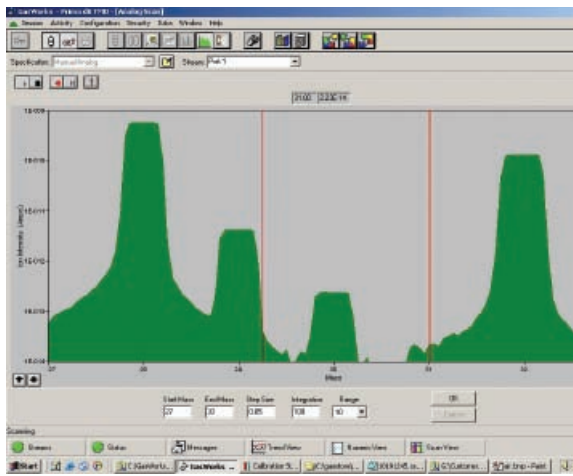


figure 4 – Spectrum of air without ethanol present.

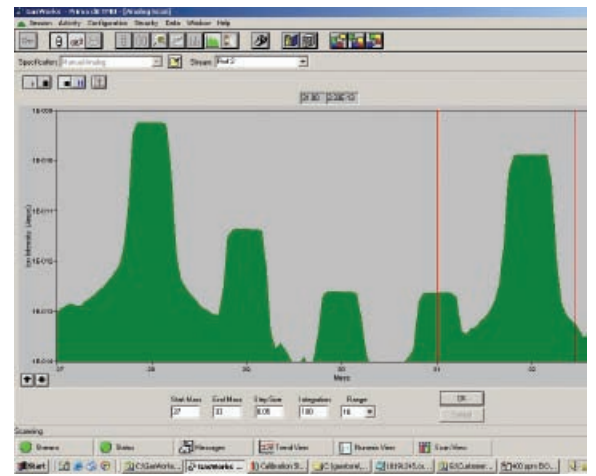


figure 5 – Spectrum of air with 400 ppm ethanol present.

of ethanol produced very different responses, indicating there were issues with ethanol adsorption in the cylinders. In order to obtain the best possible ethanol calibration, it was necessary to calibrate the Prima δ B using a vaporization device (figure 6). A vial containing ethanol liquid was placed in the temperature-controlled oven chamber.

Valco Instruments Company, Inc. offers a vial that is designed with a capillary of appropriate length and diameter to provide the required rate of ethanol evaporation through diffusion. A known flow of carrier gas flows through the oven chamber.

From 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 vapor per unit time) can be calculated, enabling accurate determination of the concentration of ethanol in the carrier gas.

The evaporation rate in terms of standard gaseous ml/min is calculated according to:

$$\text{Ethanol gaseous ml/min evaporation rate} = 22415 \times (\text{weight loss per minute})/46$$

The concentration is then calculated according to the carrier gas flow rate:

$$\% \text{ concentration of ethanol} = 100 \times \text{ethanol gaseous ml per min evaporation rate} / (\text{ethanol gaseous ml per min evaporation rate} + \text{carrier gas flow rate})$$

To check on the Prima δ B's linearity, the concentration of ethanol can be varied by adjusting the carrier gas flow rate. The concentration of ethanol is inversely proportional to the carrier gas flow rate. The linearity of the measurement (figure 7) was 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 was seen to be stable (figure 8) 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 $+55^\circ\text{C}$ causes approximately a $\pm 2\%$ relative change in ethanol concentration.

The relative standard deviation of the repeated ethanol relative sensitivity determination was $\sim 4\%$ relative. It is believed that this variation was due to weighing and flow measurement errors. However, the stability (figure 9) was still considerably better than the stability using calibration cylinders. Uncertainty in calibration was reduced by performing the calibration several times over a period of time and using an average value rather than using a single point value. The actual ethanol relative sensitivity of the Prima δ B should be very stable and, once calibrated, should not need updating more often than every six months or whenever maintenance is performed on the instrument. An example of

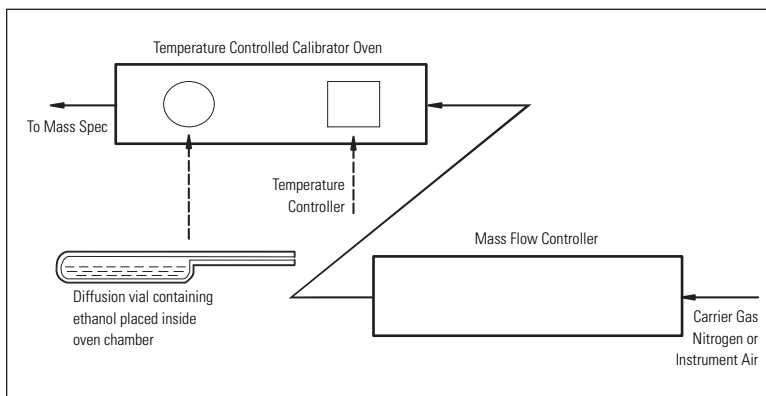


figure 6 – The setup and method for calibration of the Prima δ B using a vaporization device.

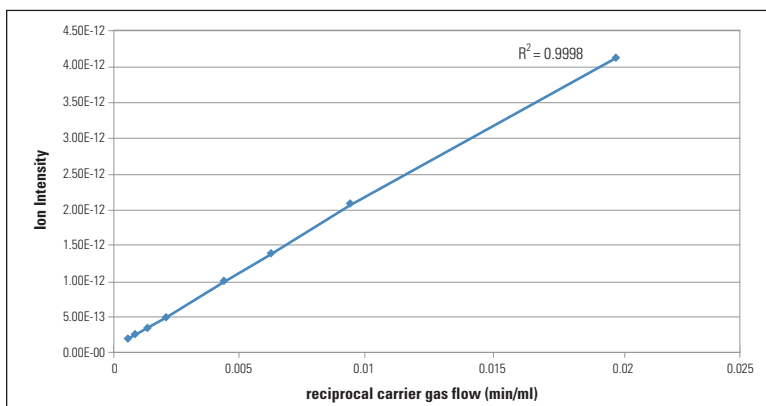


figure 7 – An example of measurement linearity using the Prima δ B tested over a flow range of 1000 ml/min down to 50 ml/min, giving a concentration range of 230 ppm up to 4600 ppm.

online analysis of ethanol in vent gas for a short batch experimental run is shown in *figure 10*.

Overview

The combination of high speed and excellent long-term stability make the Prima δB an ideal choice for continuous monitoring of ethanol production. Its magnetic sector technology enables biofuel producers to establish reliable calibration. With the Prima δB , highly reproducible, drift-free analysis for precise online measurement of ethanol is achieved without recalibration and unnecessary downtime.

References

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Stream	Name	No. of Results	Mean (Amps)	Abs SD (Amps)	% RSD
Port_2	mass31	871	2.224E-13	1.763E-15	0.79

This table represents the results of a continuous run of 230 ppm ethanol (generated by the diffusion vial) over period 15:15 – 16:45 (October 18, 2006).

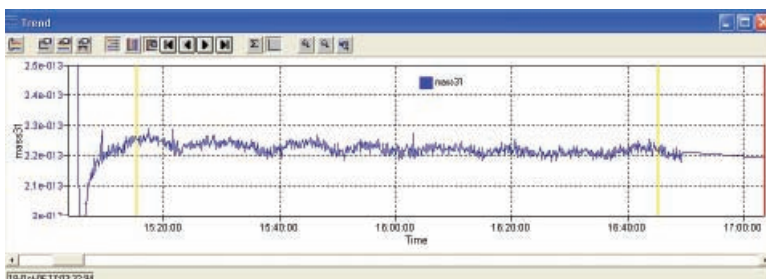


figure 8 – The chart represents the stability of the measurement of the mass 31 ion signal due to ethanol.

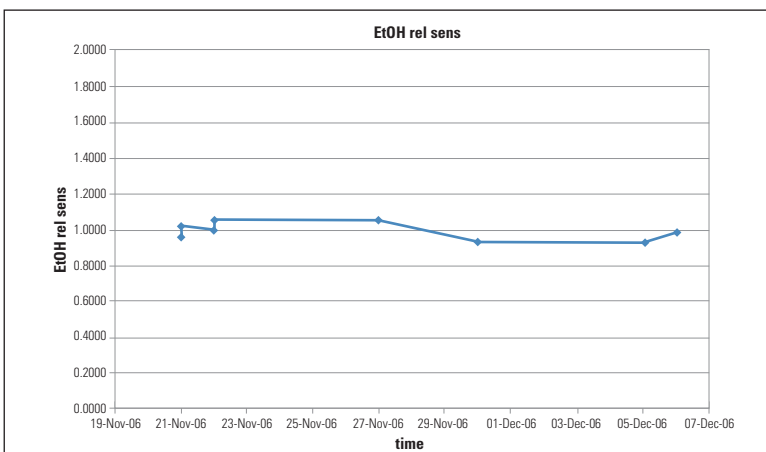


figure 9 – Data from repeated relative (to N2) sensitivity calibrations.

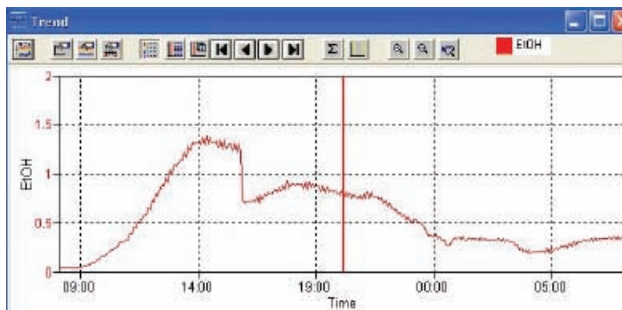


figure 10 – An example of online analysis of ethanol in vent gas.

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