APPLICATION NOTE

Prima PRO and Prima BT Process Mass Spectrometers

Quantitative analysis of bioethanol in biofuel production processes

Authors: Graham Lewis, Robert Wright,
Thermo Fisher Scientific, Winsford Cheshire UK

Keywords

- Fermentation
- Lignocellulosic Biomass
- Cellulosic Ethanol
- Thermophiles
- Magnetic Sector
- Rapid Multistream Sampler
- Carbon Dioxide Evolution
- Oxygen Uptake
- Ethanol Quantitation

Introduction

Bioethanol is the term used to describe ethanol derived from a biochemical process rather a chemical process. Initially bioethanol was 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 were concerns that using food crops to produce transport fuels would lead to competition for these feedstocks and rising food prices. A new generation of bioethanol processes has been developed that use low value, non-food based feedstocks known as lignocellulosic biomass. These includes agricultural residues, wood residues. 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. In 2017 over 27 billion US gallons of ethanol were produced worldwide, compared to 17 billion US gallons in 2007[1].

Bioethanol production

Bioethanol is produced in one of two ways. One approach is to pyrolyze 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. Whichever method is chosen to break down the biomass, there is a need to ferment sugars to produce bioethanol. The fermentation can 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

ThermoFisher SCIENTIFIC

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[2].

Analytical requirements

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 evolve. In the production of bioethanol it is also important to monitor the concentration of ethanol in the vent gas to determine the 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 so gives 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 continuously, accurately and reliably. Unlike discrete analyzers they monitor all the air gases—oxygen, carbon dioxide, nitrogen and argon. They can also monitor a wide variety of volatile organics including ethanol. Because the concentration of ethanol in the vent gas is linearly related to the concentration in the fermentor broth, they give a continuous output of the ethanol production; this is particularly important for detecting the start of ethanol production and also for monitoring changes in ethanol production.

Advantages of Thermo Scientific Prima BT and Prima PRO 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 fitted with a suitable RMS multi-stream inlet can monitor all the fermentors, in other cases separate MS analyzers have to be used in the laboratory and on 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 1 shows an example of a mass spectrometer suitable for fermentation process development, the Prima BT benchtop MS, Figure 2 shows an example of a mass spectrometer suitable for production process monitoring, the Prima PRO. Both systems use Thermo Scientific's proven magnetic sector analyzer; key advantages over alternative quadrupole analyzers include 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 on line gas analysis.

Figure 1 Prima BT process development MS

Figure 2 Prima PRO process MS

In the magnetic sector MS, ions are accelerated through a flight tube, where they are separated by their mass to charge ratios in a magnetic field of variable strength. Since the magnetic sector MS produces a focused ion beam at the detector, the peak shape obtained is 'flat-topped', i.e. uniform response is observed over a finite mass width. As the height of the peak is directly proportional to the number of ions striking the detector it is also directly proportional to the concentration of the component being measured. Provided the measurement taken at the mass of interest is on the peak's flat top, high precision analysis will be observed. If masses are aligned within the central ⅔ of the flat top region, this is normally sufficient to guard against any drift in the mass scale.

Figure 3 shows a schematic of a Prima magnetic sector MS, with the molecular ion peaks for N_2^+ and O_2^+ shown at masses 28 and 32 respectively. The flat top peak profile is seen clearly.

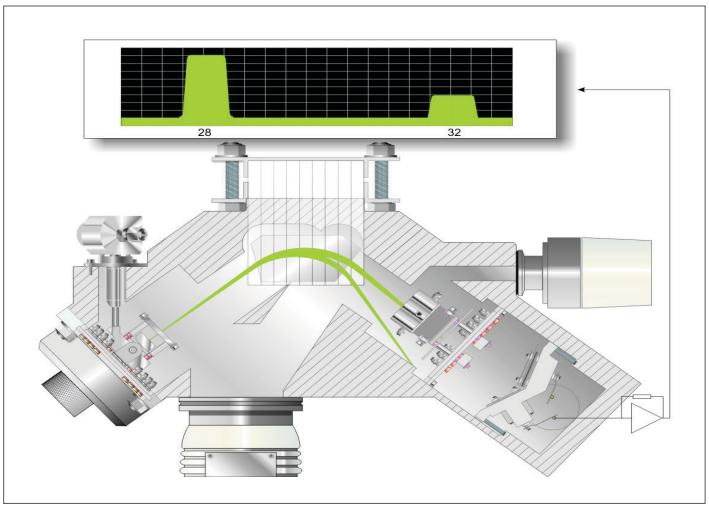

Figure 3 Magnetic sector MS schematic

Magnetic sector versus quadrupole mass spectrometers

The flat-topped peak profile of the magnetic sector MS is more 'fault-tolerant' because the measured peak heights are less influenced by misalignment or drift in the mass axis. Use of a high ion acceleration voltage to produce high energy ions in a magnetic sector instrument reduces their susceptibility to scattering by residual molecules in the vacuum system. They are also less influenced by space charge or surface charging effects due to imperfect electrode surfaces. Space charge can cause non-linear behaviour, while surface charging may cause drifting response. The quadrupole utilizes a significantly lower ion energy resulting in a rounded peak shape, and is susceptible to drift with associated lower precision and stability. Due to the less stable operation, the quadrupole calibration frequency requirement is once per week, versus monthly for magnetic sector units.

To compare quantitative performance of a magnetic sector mass spectrometer and a quadrupole mass spectrometer, representative instruments were tested for reproducibility and linearity with two different inert gas mixtures containing Helium (He, m/z 4), Argon (Ar, m/z 40), Krypton (Kr, m/z 78, 80, 82, 83, 84, and 86) and Xenon (Xe, m/z 124, 126, 128, 129, 130, 131, 132, 134, and 136). Calibration was made using Cylinder A, with 0.19% Argon, 3.9% Krypton, 38% Xenon, and balance Helium. Another cylinder, B, containing 0.10% Argon, 0.2% Krypton, 1% Xenon, and balance Helium was then analyzed. The compositions and concentrations were selected for the test on the basis that they represent both a wide mass range and concentration range and therefore are particularly challenging. The results are shown in Table 1. The column titled '% Rel Diff' shows the % relative difference between the mean measured concentration and the cylinder certificate concentration (i.e., accuracy). The stability of the analysis is represented by the column titled 'Std Dev' which is standard deviation of 30 repeated measurements.

Table 1 Comparison of stability and accuracy of magnetic sector and quadrupole mass spectrometers

	Cert mol%	Mean mol%	% Rel Diff	Std Dev mol%	% Rel Std Dev	Cert mol%	Mean mol%	% Rel Diff	Std Dev mol%	% Rel Std Dev
	Magnetic sector mass spectrometer									
He	57.9321	57.8853	-0.08	0.0182	0.03	98.688	98.6098	-0.08	0.0007	0.00
Ar	0.1899	0.1883	-0.83	0.0002	0.12	0.102	0.0972	-4.71	0.0002	0.17
Kr	3.876	3.8663	-0.25	0.0017	0.04	0.2	0.2030	1.48	0.0002	0.12
Xe	38.002	37.9027	-0.26	0.0167	0.04	1.01	1.0168	0.67	0.0007	0.07
	Quadrupole mass spectrometer									
He	57.9321	58.3334	0.69	0.2383	0.41	98.688	98.4114	-0.28	0.0168	0.02
Ar	0.1899	0.1817	-4.34	0.0012	0.66	0.102	0.1025	0.47	0.0008	0.82
Kr	3.876	3.7872	-2.29	0.0174	0.46	0.2	0.2308	15.38	0.0022	0.95
Xe	38.002	37.5854	-1.10	0.2195	0.58	1.01	1.1857	17.39	0.0129	1.09

The standard deviation values for the two instruments for cylinders A and B, and accuracy values for cylinder B obtained at different concentrations, including the separate isotopes of Krypton and Xenon, are shown in Figures 4 and 5 respectively. It is seen that the level of performance for stability and accuracy is about 10 times better for the magnetic sector instrument compared with the quadrupole type.

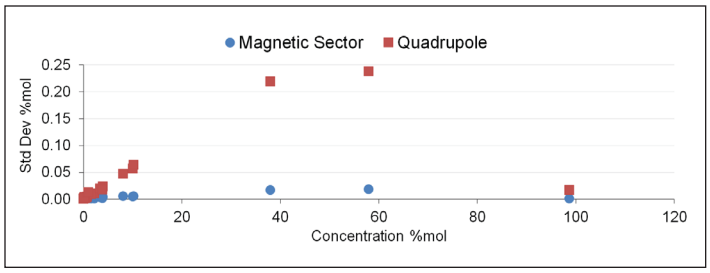

Figure 4 Standard Deviations versus Concentrations in cylinders A and B (all components)

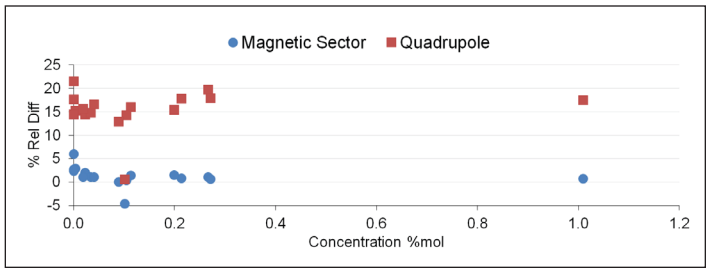

Figure 5 Accuracy versus Concentrations in cylinder B (Argon, Krypton, Xenon components)

Table 2 Typical magnetic sector MS performance specification for fermentation

Measurement (% mol)	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*

Thermo Scientific GasWorks software permits analysis optimization on a per-stream basis so we can select the most appropriate speed versus precision setting depending on process control requirements. We can also set up different analyses on different sample points—for example, analyze air gases in the inlet sparge stream and air gases plus ethanol in the outlet streams. Similarly we can select the most efficient peak measurements for each stream and the most appropriate display units (% or ppm).

An example of data obtained from a Prima BT magnetic sector MS is shown in Figure 6. The data are from a routine stability test measuring reference air over one week without re-calibration. Oxygen readings are stable to within ±0.01 mol%, while Carbon Dioxide readings are stable to within ±5 ppm.

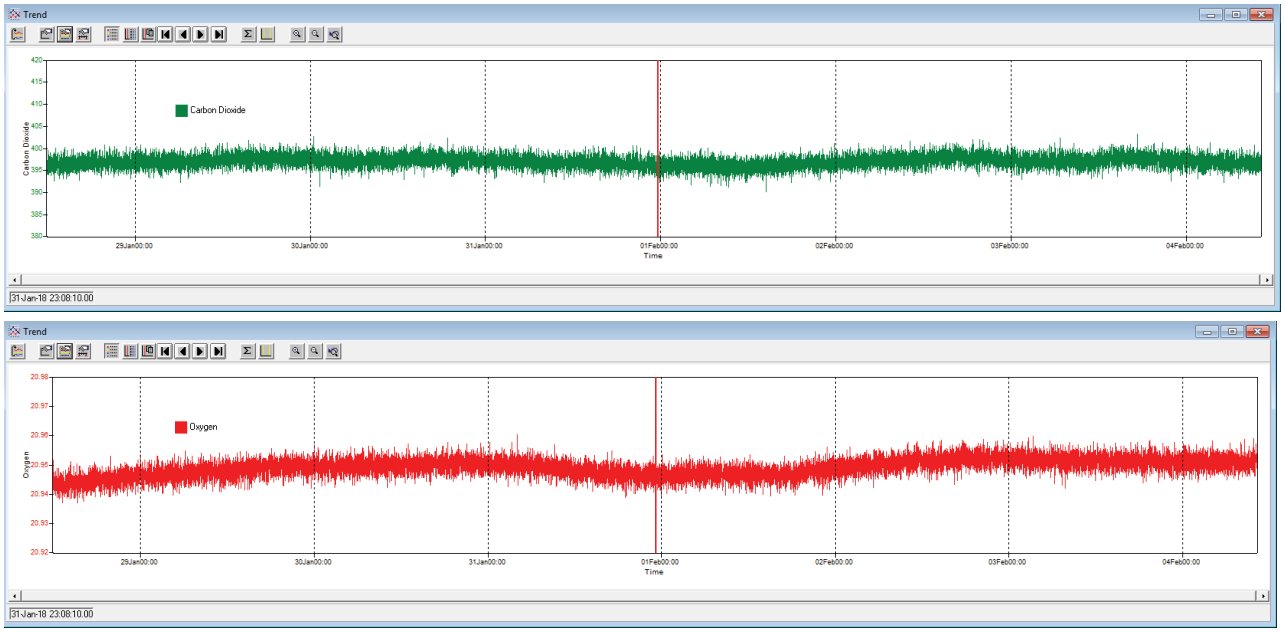

Figure 6 Prima BT stability test measuring reference air over one week without re-calibration

Ethanol analysis

The accurate online analysis of ethanol is essential to understand the fermentation process kinetics and to close the mass balance. Figure 4 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.

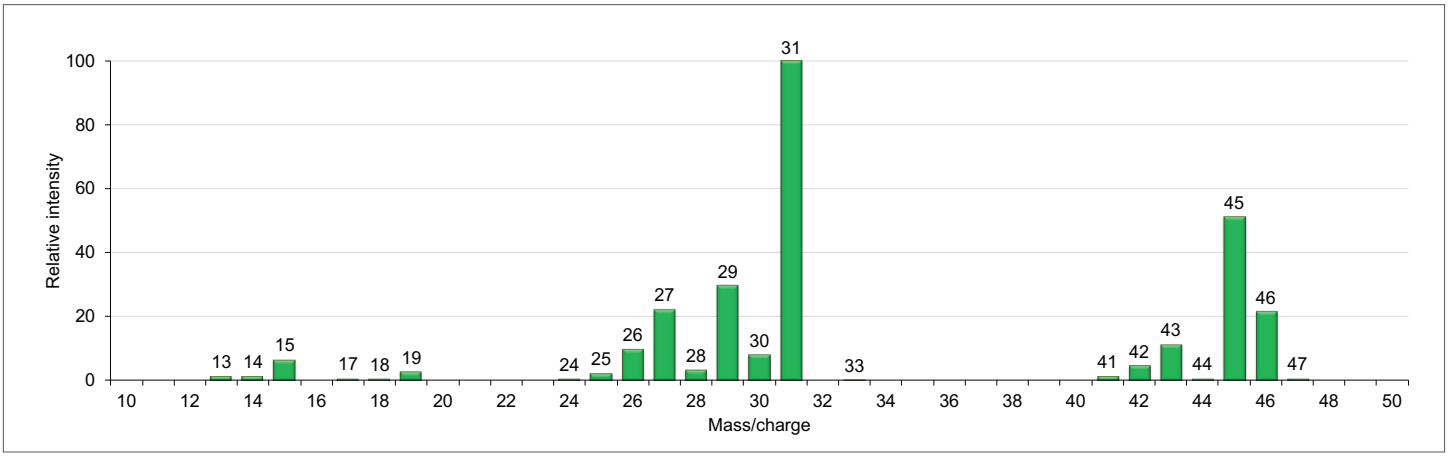

Figure 7 Ethanol fragmentation pattern

However 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 the 32 peak 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 accordingly corrected. On a quadrupole instrument this interference level is much greater and also variable, resulting in excessive uncertainty in low level ethanol measurement. A low level ethanol signal effectively tends to get 'buried' in the noise from the oxygen peak.

With Prima's magnetic sector instrument the measurement is very reproducible and ethanol can be measured with a precision down to 10 ppm. Figure 8 shows the mass spectrum around mass 31 for air with no ethanol present, Figure 9 shows the logarithmic spectrum for air containing around 400 ppm ethanol.

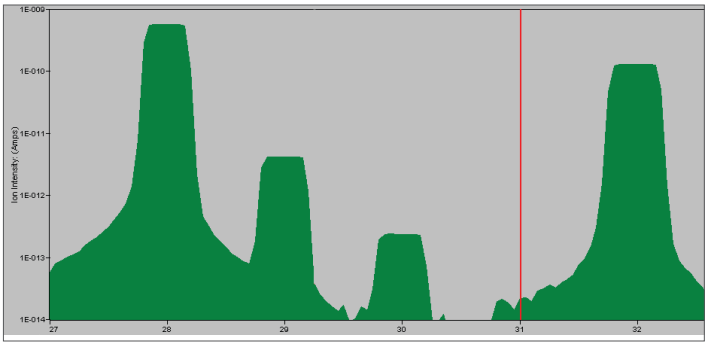

Figure 8 Spectrum of air without ethanol

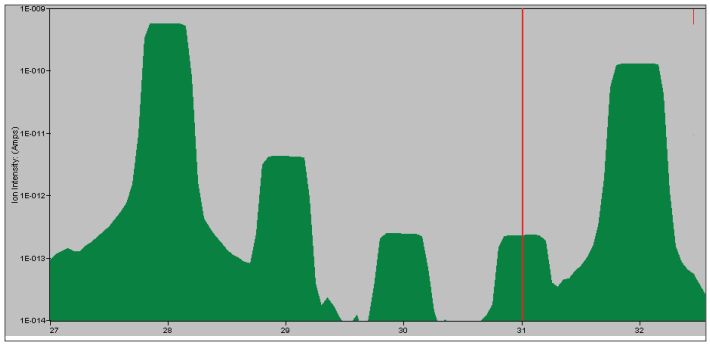

Figure 9 Spectrum of air with 400 ppm ethanol

Ethanol linearity

Table 3 and Figure 10 show the results of an ethanol linearity study with Prima BT, which was calibrated for ethanol using a 400 ppm ethanol in balance nitrogen cylinder, and a 15% oxygen, 5% carbon dioxide, 1% argon, and balance nitrogen cylinder to correct for the oxygen (m/z 32) interference on m/z 31. The ethanol linearity tests were performed with different concentrations of ethanol (100 – 1000 ppm) in cylinders containing 10% oxygen, 5% carbon dioxide, 1% argon, and balance nitrogen. Ethanol linearity was demonstrated in the presence of oxygen with Prima BT ethanol measurements showing close agreement to calibrated cylinder values (maximum % difference = 6%).

Table 3 Prima BT Ethanol Linearity Results (100 – 1000 ppm)

Actual ethanol concentration ppm	Prima BT reading	% Difference from expected
(Certificate accuracy ±2%)*	ppm	0.4
96.4	96.8	2.7
257	263.9	0.2
492	493.2	6.1
1046	1110	2% relative or 0.001 %mol*

** All ethanol calibration cylinders contain 10% oxygen, 5% carbon dioxide, 1% argon, and balance nitrogen*

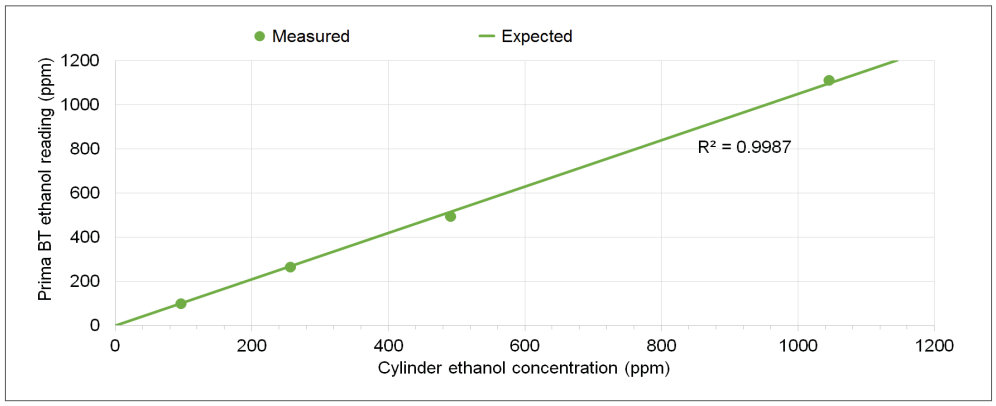

Figure 10 Prima BT ethanol linearity results comparing calibration cylinder to MS measurements

Ethanol response

Apart from interference, another effect seen with ethanol is the memory effect. Ethanol tends to adsorb on surfaces, particularly in the high vacuum system of a mass spectrometer ion source. This causes a delay in response, leading to problems with the ethanol readings:

- Switching from low to high ethanol the reading is too low, because the signal is measured before it has had time to stabilize (i.e. ethanol build-up effect)
- Switching from high to low ethanol the reading is too high, because some ethanol from the previous sample is still present (i.e. ethanol settling effect)

These effects can result in inaccurate ethanol measurements and much slower/less frequent measurements, both of which compromise the analytical performance. Operating the ion source at a higher temperature improves the response. However the most significant improvement has been obtained by replacing stainless steel surfaces with glass, particularly at the gas entrance to the ion source. Within the ion source, glass cannot be used to replace stainless steel surfaces because all the electrode surfaces need to be conducting.

The ion source block of the Thermo Scientific Prima BT is represented in Figure 11. The inlet gas passes through a small orifice tube to the side of the stainless steel ion source via a channel (shown in yellow) to the ionization region. Making this channel out of glass rather than stainless steel considerably improves the response to ethanol.

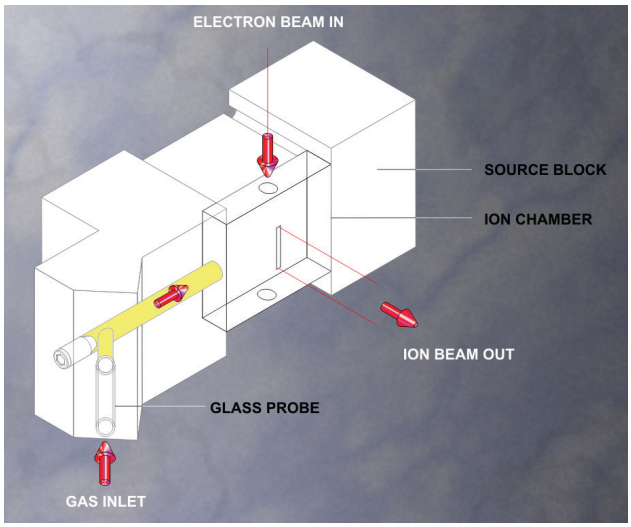

Figure 11 Redesigned ion source with glass lined entrance (highlighted in yellow)

Figure 12 shows the ethanol build-up and settling profiles for a stainless steel lined ion source and a redesigned ion source with glass lined entrance, based on measurements from a 100 ppm ethanol in nitrogen balance gas cylinder. The ethanol build-up profile was determined by switching from a valve with no ethanol (i.e. air stream) to a valve with a constant ethanol concentration (i.e. 100 ppm ethanol in nitrogen balance gas cylinder), and allowing the ethanol concentration to reach a steady state (maximum) value. The ethanol settling profile was determined by switching from a valve with constant ethanol concentration (i.e. 100 ppm ethanol in nitrogen balance gas cylinder) to a valve with no ethanol (i.e. air stream), and allowing the ethanol concentration to decrease to below the ethanol detection limit (10 ppm).

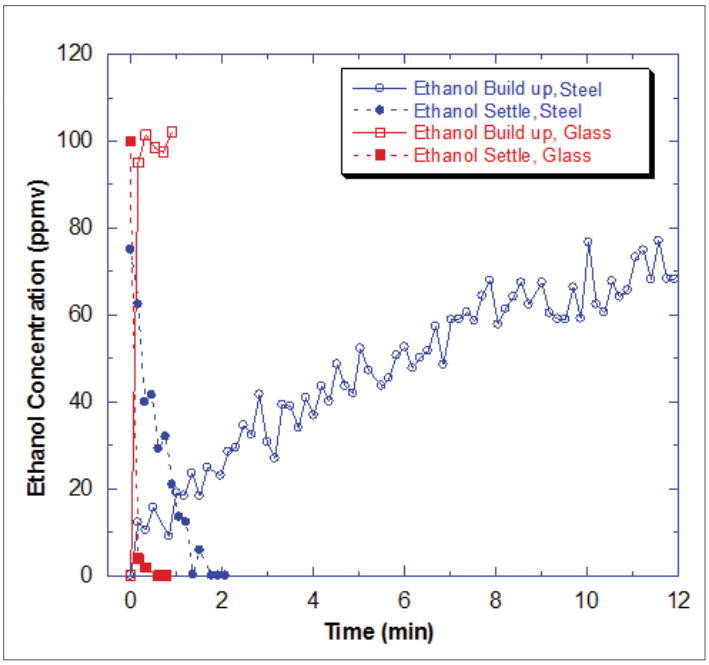

Figure 12 Ethanol build-up (open markers, solid lines) and settling (solid markers, dashed lines) for stainless steel ion source (blue lines) and redesigned ion source with glass lined entrance (red lines) based on a 100 ppm ethanol in balance nitrogen gas cylinder

resulted in significant reductions in ethanol detector build-up and settling times versus the original ion source. The ethanol build-up time was reduced from 12 minutes with the stainless steel source design to less than one minute with the glass lined entrance, and ethanol settling time was reduced from 90 seconds to less than 20 seconds. The steady state ethanol concentration reached after 12 minutes with the stainless steel source was 70 ppm, considerably lower than the expected 100 ppm value obtained with the glass lined entrance.

The ethanol absorption effect resulting in long ethanol build-up time and lower than expected ethanol measurement seems to be enhanced at the lower ethanol concentration range.

Figure 13 shows the ethanol build-up and settling profiles based on a 400 ppm ethanol in nitrogen balance gas cylinder. The ethanol build-up time with the stainless steel source was 5 minutes with 400 ppm, compared to 12 minutes with 100 ppm ethanol, and the steady state concentration reached was the expected value of 400 ppm ethanol. At 400 ppm the settling time with the stainless steel source was more than 4 minutes, compared to less than 30 seconds with the glass lined entrance. The glass lined entrance implementation has a significant impact on reducing valve delay time and increasing sample frequency, and improving accuracy for lower ethanol concentration range (< 100 ppm ethanol).

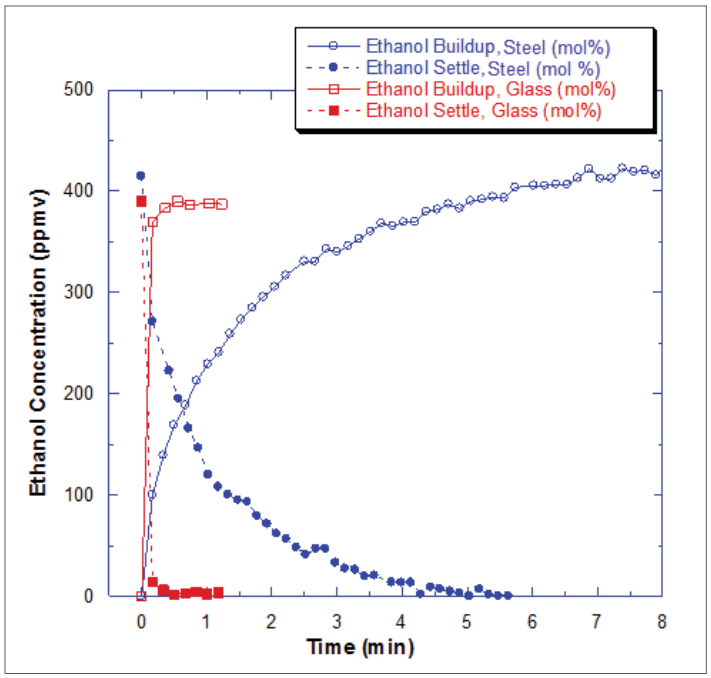

Figure 13 Ethanol build-up (open markers, solid lines) and settling (solid markers, dashed lines) for stainless steel ion source (blue lines) and redesigned ion source with glass lined entrance (red lines) based on a 400 ppm ethanol in balance nitrogen gas cylinder

thermo scientific

Summary

The 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 Prima BT in the development laboratory or Prima PRO in the production plant. They have been used on a wide range of biotechnology processes, from biopharmaceuticals to biomaterials to biofuels

Many of these processes require fast, accurate analysis of ethanol. Implementation of a modified ion source with glass lined entrance resulted in accurate quantitation of low concentration ethanol (20–100 ppm), and a significant increase in bioreactor sampling frequency due to reduced ethanol build-up and settling times. Online measurement with Prima BT and Prima PRO provides:

- Linear, accurate measurement of ethanol over wide concentration range (20–1000 ppm)
- Accurate carbon dioxide evolution and oxygen uptake quantitation for process understanding and optimization
- Reduced process development time and effective optimum ethanol production strain identification
- Fast measurement for increased sampling frequency from multiple reactors
- Significant reduction of off-line sampling requirements
- Reduced calibration frequency requirements
- Fault tolerant designs combined with extended intervals between maintenance and simplified maintenance procedures ensure maximum availability with normal uptime > 99.8%.

Acknowledgments

We wish to thank Kevin Hettenbach (now of Gingko Bioworks) and Kari Paisley-Flango (now of Moderna Therapeutics) for their contribution to the work on testing the new ion source design.

References

1. Renewable Fuels Association website, World Fuel Ethanol Production
2. Optimization of next generation bioethanol production, Hydrocarbon Engineering June 2008, Peter Traynor, Robert Wright and Neil Conroy

USA
27 Forge Parkway
Franklin, MA 02038
Ph: (800) 437-7979
Fax: (713) 272-2273
orders.process.us@thermofisher.com

India
C/327, TTC Industrial Area
MIDC Pawane
New Mumbai 400 705, India
Ph: +91 22 4157 8800
india@thermofisher.com

China
+Units 702-715, 7th Floor
Tower West, Yonghe
Beijing, China 100007
Ph: +86 10 84193588
info.eid.china@thermofisher.com

Europe
Ion Path, Road Three,
Winsford, Cheshire CW73GA UK
Ph: +44 1606 548700
Fax: +44 1606 548711
sales.epm.uk@thermofisher.com

Find out more at **thermofisher.com/primapro**

ThermoFisher SCIENTIFIC