

# Fermentation Off Gas Analysis Using the VG Prima $\delta$ B

## Key Words

- Mass Spectrometry
- Fermentation
- Respiratory Quotient
- Volatile Organics
- Off Gas Analysis
- Biotechnology
- Pharmaceutical

## Thermo Industrial Solutions Note

### Introduction

Biotechnology has led to the discovery and development of a new generation of human therapeutics, improved agriculture products, new sources of renewable energy and novel bio-degradable materials. Advancements in cellular and molecular biology have contributed to therapies where previously there was no effective treatment.

In the field of bio-agriculture, experts assert that biotechnology innovations will triple crop yields without requiring any new farmland being brought into production, saving valuable rain forests and animal habitats. The new biomaterial industry promises energy efficient production of plastics without unnecessary consumption of finite fossil fuels.

One of the most important biotechnological processes is fermentation. This note details

the application of the VG Prima  $\delta$ B Process Mass Spectrometer in monitoring the headspace of fermentors and bioreactors.

### Fermentation

Fermentation is the term used by microbiologists to describe the production of a product by means of the mass culture of a micro-organism. This product can either be the cell itself (biomass production), the micro-organism's own metabolite or a foreign product. Micro-organisms that carry out their metabolism using oxygen are referred to as aerobic micro-organisms. Some micro-organisms can substitute nitrate or sulfate for oxygen and thus grow in the absence of oxygen. These micro-organisms are referred to as anaerobic.

There are three variations of the fermentation process. In batch fermentation, a sterilized nutrient solution in the fermentor is inoculated with micro-organisms and incubation is allowed to proceed. During



VG Prima  $\delta$ B Scanning Magnetic Sector Process Mass Spectrometer

the course of the fermentation, oxygen is added (in case of aerobic micro-organisms) and acid or base to control the pH. The composition of the culture medium, the biomass concentration, and the metabolite concentration generally change constantly as a result of cell activity. An enhancement of the closed batch process is the fed-batch fermentation, where substrate is added in increments as the fermentation progresses.

In continuous fermentation, an open system is set up and sterile nutrient solution is added to the bioreactor continuously. An equivalent amount of converted nutrient solution with micro-organisms is simultaneously harvested off the system.

A typical fermentor is shown in schematic form in *figure 1*. A microbial fermentation can be viewed as a three-phase system, involving liquid-solid, gas-solid,

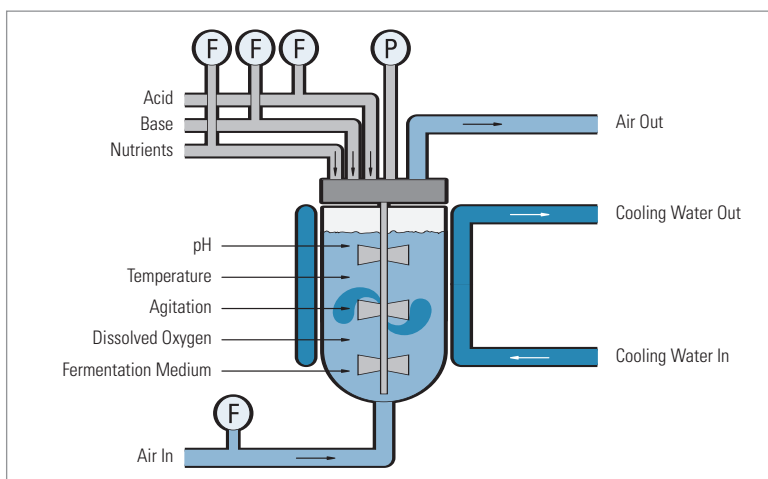


figure 1 – Typical Fermentor — schematic

and gas-liquid reactions.

The liquid phase contains dissolved nutrients, dissolved substrates and dissolved metabolites.

The solid phase consists of individual cells, pellets, insoluble substrates, or precipitated metabolic products.

The gaseous phase provides a reservoir for O<sub>2</sub> supply and CO<sub>2</sub> removal.

### Need for Gas Analysis

In any fermentation, it is essential to monitor the state of the culture, since its health determines the conversion rate of nutrients, the formation of unwanted by-products and, in worst case, the onset of poisoning. Analysis of the respiratory gases being fed to and removed from the fermentor is an ideal means of characterizing the fermentation. It is non-invasive and enables monitoring of the physiological state of the fermentation, including growth kinetics and substrate consumption. It also helps determine the optimum point to halt the process for maximum yield.

### Why Use Process MS?

Many fermentations are characterized by small changes in oxygen and carbon dioxide concentrations at critical phases

$$CER (CO_2 \text{ Evolution Rate}) = \%Volume \text{ of } CO_2 \text{ out} \times flow \text{ out} - \%Volume \text{ } CO_2 \text{ in} \times Flow \text{ in}$$

$$OUR (O_2 \text{ Uptake Rate}) = \%Volume \text{ of } O_2 \text{ in} \times flow \text{ in} - \%Volume \text{ } O_2 \text{ out} \times Flow \text{ out}$$

$$RQ (\text{Respiratory Quotient}) = CER/OUR$$

table 1 – Fermentation Off Gas Analysis and Respiratory Quotient

of the fermentation, for example, during the lag phase when the micro-organisms exist in equilibrium with the nutrients.

It is vital that the method used for measuring off gas is capable of fast, precise analysis. The speed of MS makes it ideal for the application and the high precision of the scanning magnetic sector MS in the VG Prima δB has made it the pre-eminent process gas analyzer in fermentation off gas analysis.

### Respiratory Quotient

Respiration is the process whereby an organism oxidizes food to produce energy. An important control parameter in the fermentation process is the Respiratory Quotient (RQ). This is the ratio of the carbon dioxide evolution rate (CER) to the oxygen uptake rate (OUR). The full calculation of RQ is shown in table 1. The accurate

determination of RQ relies on determination of the ratio of the flows in and out of the fermentor.

This ratio is easily determined by a scanning MS, which can measure N<sub>2</sub> and Ar in addition to O<sub>2</sub> and CO<sub>2</sub>. At least one of these two gases will be inert to the process so it can be used effectively to correct for the humidity change that occurs when the dry air feed gas is bubbled through the fermentor liquid. Without this correction, errors are introduced into the headspace data due to dilution by the additional water vapor

VG GasWorks software calculates RQ as a standard feature for the fermentation application.

Results from an E. coli fermentation monitored over 6 hours by the VG Prima are shown in figure 2.

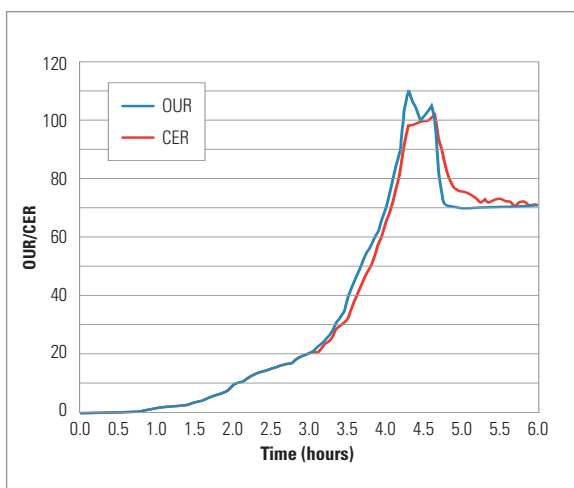


figure 2 – Oxygen Uptake Rate (OUR) and Carbon Dioxide Evolution Rate (CER) for E. Coli fermentation using the VG Prima δB

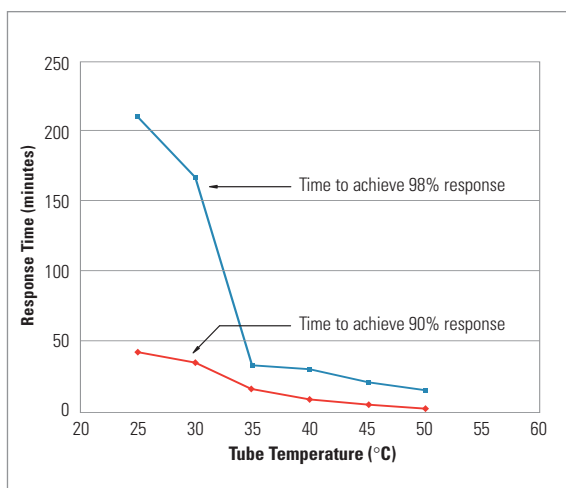


figure 3 – Effects of sample line temperature on response to ethanol. Data courtesy of Smith Kline Beechams

## Analysis of Volatiles

The respiratory gases are not the only species of interest in the off gas. Volatile organics such as methanol, ethanol, ethyl acetate and diacetyl are found at ppm levels in the headspace and their analysis can yield vital information on the well-being of the fermentation. However, their analysis provides certain technical problems that must be overcome if the analytical data is to be meaningful. These issues are discussed below.

## Description of Analyzer

The VG Prima  $\delta$ B magnetic sector mass spectrometer utilizes technology which has been proven over many years in a wide range of industrial applications, including biotechnology, petrochemicals and iron and steel. The method of analysis is achieved using the following components.

### Sample inlet system

The inlet system consists of a rapid multi stream (RMS) rotating sampler. Continuous sample flow and zero dead volume eliminate the need for long sample flushing times. Constant inlet position feedback and gas flow rate monitoring provide ultimate sample integrity. The only consumable component is a sample seal, which typically completes over 5 million operations between servicing. This enables a VG system to sample up to 60 fermentors.

For the successful analysis of volatiles, it is important that both the sample lines and the inlet systems are heated to avoid unacceptably long response times. *Figure 3* shows the effect of temperature of PTFE sample lines on system response to ethanol. The RMS can be heated to maximum 120°C ensuring fast response to even the most 'sticky' of volatiles.

## Analyzer

There are essentially two types of mass spectrometers available for continuous gas analysis. Thermo Electron Corporation is unique in that we offer both types of MS systems. Magnetic sector has proven the most successful for fermentation off gas analysis due to the inherently superior stability and lower maintenance requirements.

Gas components are separated according to their individual molecular weights. The gas molecules are ionized in a high vacuum by electronic bombardment. The pressure of the sample is reduced to the required vacuum level in a two stage process, a capillary, followed by a molecular leak into the ion source. This gives excellent dynamic range of measurement (ppm to percent concentrations are analyzed quite easily). In addition, the capillary and inlet are glass lined and heated, to ensure fast response to volatiles such as alcohol.

The positive ions formed in the ion source are then accelerated through a magnetic field. The strength of the magnetic field is varied and this allows the instrument to select different gas species. The resulting highly stable mass spectra can then be measured to determine the composition of the gas mixture. The measurement device is a Faraday collector coupled with a fixed gain amplifier. Frequent electronic zeroing eliminates baseline drift and reduces the calibration frequency.

The alternative technique is quadrupole mass spectrometry, which separates masses according to their mass to charge ratios by accelerating the positive ions between four rods with an RF/DC potential of varying power. However, this method has some significant disadvantages compared with the magnetic sector analyzer.

Quadrupole analyzers operate at ion energies of typically 5 volts. This can give rise to ion interactions within the source causing poor short-term precision. The quadrupole analyzer is therefore inadequate for measuring RQ with the required precision and stability.

The signal intensity at any specific mass position on a magnetic sector analyzer appears as a 'flat top peak.' This means that any small drift in the mass scale will not result in a change in signal intensity. This is not the case with quadrupole mass spectrometers that provide rounded, shaped peaks.

The standard performance specifications for the VG Prima  $\delta$ B is shown in *table 2*. Precision is the standard deviation observed over 24 hours. Note the extremely high precision — 0.05% relative over 24 hours for oxygen.

	Sample Gas (% Molar Conc.)	Precision % Absolute
N <sub>2</sub>	79	0.008
O <sub>2</sub>	15	0.008
Argon	1	0.002
CO <sub>2</sub>	5	0.005
Methanol	0.004	0.001
Ethanol	0.004	0.001

table 2 – VG Prima Standard Performance Specifications

## Sample Conditioning

A typical sample conditioning system is shown in *figure 4*. The VG Prima  $\delta$ B requires a sample flow of 0.1-1 l/min at atmospheric pressure, which should be verified by rotameter. To protect the analyzer against blockage by liquids and particulates, we recommend the use of a two filter system.

This system is typically a Balston filter followed by a membrane filter (e.g. Genie Membrane filter Model 101 high flow). A foam trap may be added if necessary.

## Summary

The VG Prima series Magnetic Sector Process MS has now been successfully monitoring fermentor off-gas at many of the world's leading biotechnology and pharmaceutical companies for many years. By combining high speed with excellent stability, the magnetic sector analyzer lends itself ideally to this demanding application.

The VG Prima  $\delta$ B from Thermo Electron benefits from being housed in a rugged, industry standard enclosure and has the advantages of the unique Rapid Multi Stream inlet system, GasWorks Windows<sup>®</sup> based software for flexibility and ease of use and a host of plant interfacing technologies.

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## For More Information

Thermo Electron Corporation  
Process Instruments  
North American Office  
9303 W. Sam Houston Pkwy. S.  
Houston, TX 77099 USA  
(877) 290-7422

Thermo Electron Corporation  
Process Instruments  
European Office  
Ion Path, Road Three  
Winsford, Cheshire CW7 3GA  
United Kingdom  
+44 (0) 1606 548700

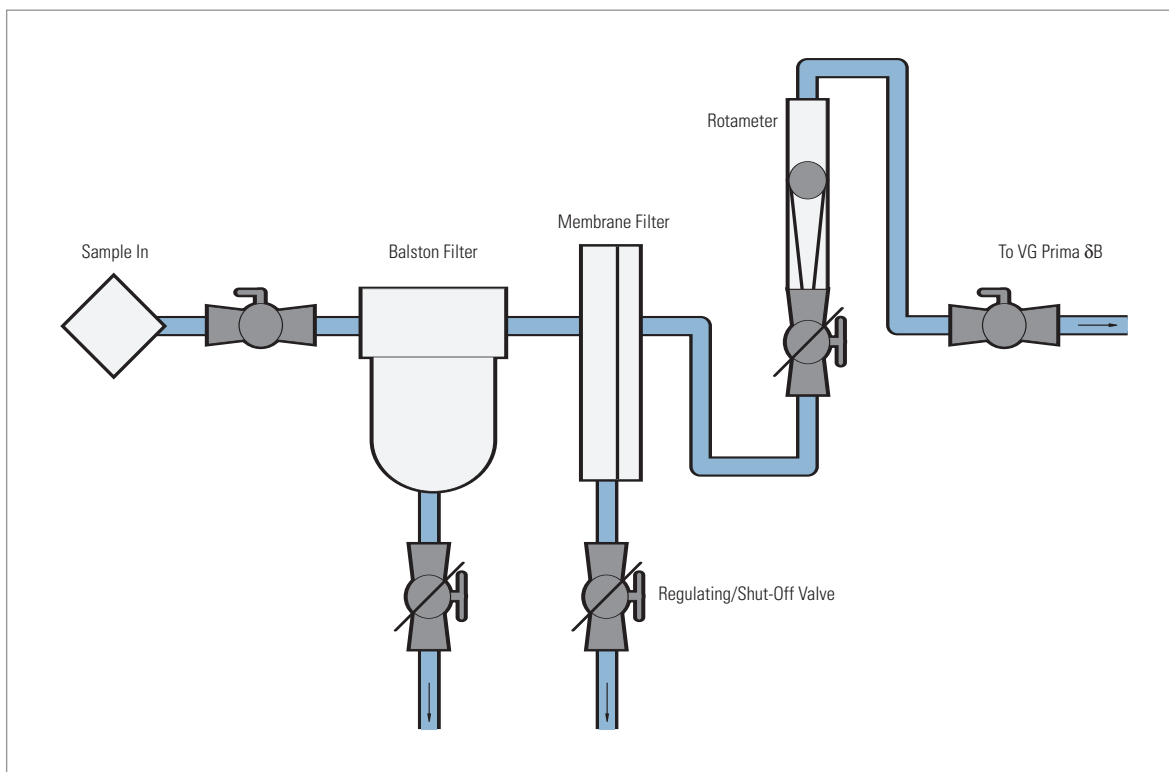


figure 4 – Typical Sample Conditioning for Fermentor Off Gas Analysis