

# Gas Analysis Mass Spectrometry Applications in Fermentation and Cell Culture Processes

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## Key Words

- Biotechnology
- Fermentation
- Off-gas Analysis
- Rapid Multistream Sampling
- Magnetic Sector
- Oxygen Uptake Rate
- Carbon Dioxide Evolution Rate
- Respiratory Quotient

## Introduction

The fermentation process is used to produce a wide range of key products in a variety of industries:

**Pharmaceuticals:** antibiotics, vaccines, prophylactics, hormones

**Bioenergy:** bio-alcohol fuels based on low value, non-food based feedstocks

**Biomaterials:** energy efficient, bio-degradable plastics

**Animal nutrition:** feed supplements, amino acids

Other important fermentation products include industrial enzymes, food additives and vitamins.

By definition, the term 'fermentation' refers to anaerobic processes (those that take place without the presence of oxygen). If oxygen is present, the process is aerobic and should be called 'respiration'. However, in biotechnology, 'fermentation' is used more loosely to refer to the growth of microorganisms on food, in either aerobic or anaerobic conditions. The latter definition will be used throughout this paper.

## Fermentation

Fermentation is the term used by microbiologists to describe the production of a compound by means of the mass culture of a microorganism. This product can either be the cell itself (biomass production), the microorganism's own metabolite or a foreign product. Microorganisms that carry out their metabolism using oxygen are referred to as aerobic microorganisms.



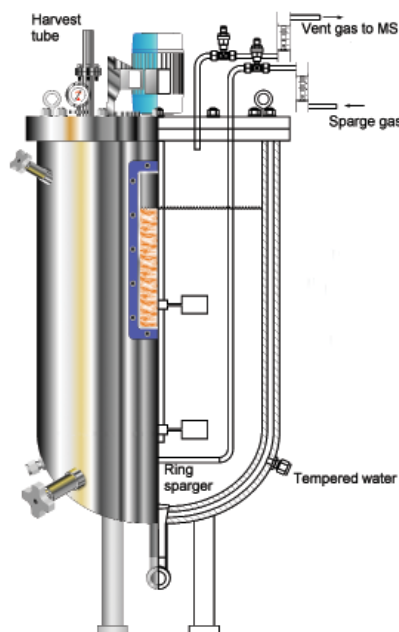
Some microorganisms can substitute nitrate or sulfate for oxygen and thus grow in the absence of oxygen. These microorganisms are referred to as anaerobic.

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 the course of the fermentation, oxygen is added (as in the case of aerobic microorganisms) and an 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 microorganisms is simultaneously harvested off the system.

A microbial fermentation can be viewed as a three-phase system, involving liquid-solid, gas-solid, 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 oxygen supply and carbon dioxide removal.

A typical pilot scale fermentor is shown in schematic form in Figure 1.



**Figure 1:** Typical pilot scale fermentor.

### The 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 the worst case, the onset of poisoning. Analysis of the respiratory gases being fed into and removed from the fermentor is an ideal way 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.

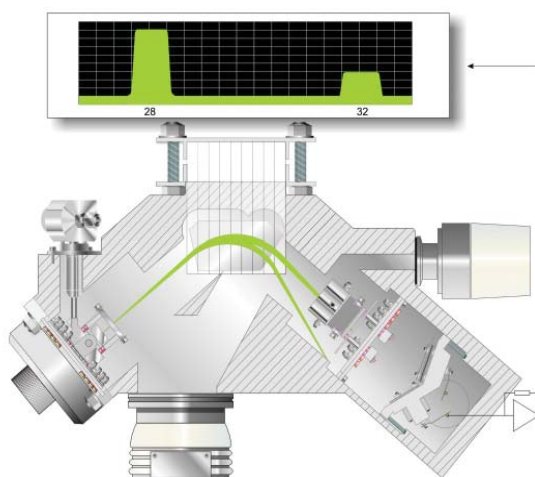
### Why Use Mass Spectrometry for Gas Analysis?

Many fermentations are characterized by small changes in oxygen and carbon dioxide concentrations at critical phases of the fermentation, such as the lag phase when the microorganisms exist in equilibrium with the nutrients. It is vital that the method used for measuring off-gas is capable of fast and precise analysis. The speed of mass spectrometry (MS) makes it ideal for the fermentation application. However, speed must not be at the expense of precision; it is equally important that precise data is acquired so that small changes in concentration are not lost.

### Advantages of Magnetic Sector MS

Two types of MS have been used to monitor fermentation processes: magnetic sector, where charged particles are separated in a variable magnetic field, and quadrupole, where charged particles are separated in a variable RF field. 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 fermentation off-gas analysis<sup>1,2</sup>.

Key advantages of magnetic sector 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. A schematic of a Magnetic Sector mass spectrometer analyzer is shown in Figure 2.



**Figure 2:** Schematic of Magnetic Sector MS.

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 peaks. The magnetic sector analyzers used in the Thermo Scientific™ Prima family of mass spectrometers are laminated, so they scan at speeds equivalent to that of quadrupole analyzers, offering the unique combination of rapid analysis and high stability. This allows the fast and extremely stable analysis of an unlimited number of user-defined gases.

### Rapid Multistream Sampling

If the MS is to monitor multiple fermentors, then a fast and reliable means of switching between streams is required. Solenoid valve manifolds have too much dead volume and rotary valves suffer from poor reliability so we developed the unique Rapid Multistream Sampler (RMS). It offers an unmatched combination of sampling speed and reliability and allows sample selection from

up to 64 streams. Stream settling times are application dependent and completely user configurable. The RMS includes digital sample flow recording for every selected stream. This can be used to trigger an alarm if the sample flow drops, for example if a filter in the sample conditioning system becomes blocked. The RMS is heated to ensure fast response to even the most 'sticky' of volatiles.

## 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) (Table 1).

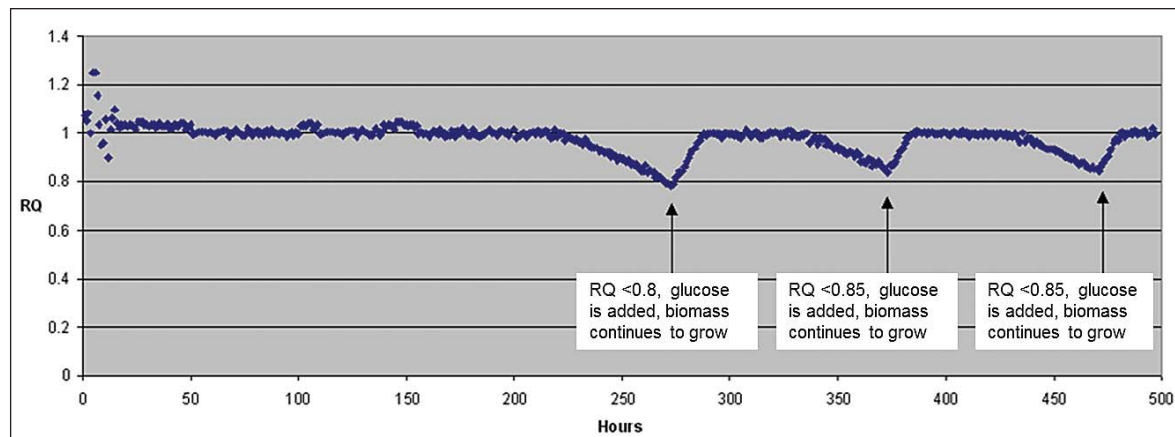
Parameter	Calculation
CER	= (%Volume CO <sub>2</sub> out x Flow out) - (%Volume CO <sub>2</sub> in x Flow in)
OUR	= (%Volume O <sub>2</sub> in x Flow in) - (%Volume O <sub>2</sub> out x Flow out)
RQ	= CER/OUR

**Table 1:** Parameters and respiratory quotient for fermentation off-gas analysis.

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<sup>3</sup>. The calculation for RQ using nitrogen as the flow correction is shown in Equation 1. The Thermo Scientific Gas-Works software calculates RQ as a standard feature for the fermentation application.

$$RQ = \frac{\{CO_2\text{out} \times (N_2\text{in} / N_2\text{out})\} - CO_2\text{in}}{O_2\text{in} - \{O_2\text{out} \times (N_2\text{in} / N_2\text{out})\}}$$

**Equation 1:** Respiratory Quotient calculated by MS



**Figure 3:** Respiration Quotient (RQ) data generated by MS from fed-batch fermentation.

Figure 3 shows an example of using RQ values measured by the mass spectrometer to trigger glucose additions to maximize viable cell density in a fed-batch fermentation. In this example the nutrient mix is designed to provide optimum nutrition for approximately 250 hours in order to ensure rapid increase in cell density. Once the mass spectrometer indicates that the RQ has fallen below 0.8 as the last of the glucose is consumed, then glucose is added so the biomass can continue to grow. The next control point is set a little higher at 0.85 when the second glucose addition is triggered. This degree of control can only be provided with a very precise RQ measurement. No other technique can match this level of precision. It is worth noting that the precision is lower at the very start of the fermentation since the volume of oxygen consumption is extremely low and the signal to noise ratio is correspondingly low. This period is termed the lag phase during which the cell count is extremely low. Once the organisms begin to multiply, the precision quickly improves.

## 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 even hydrogen sulfide can be 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.

For example the measurement of trace levels of methanol and ethanol require the measurement of the CH<sub>2</sub>OH<sup>+</sup> fragment at mass 31. However we need to consider the presence of a very large peak at mass 32 from the percentage levels of O<sub>2</sub> in the vent gas. We need to correct for the tail from the 32 peak if we are to make an accurate measurement of trace alcohol levels.

The intensity of the tail from O<sub>2</sub> at mass 31 compared with the intensity of the peak at mass 32 is 0.02%.

When the concentration of O<sub>2</sub> 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 properly corrected.

On a quadrupole instrument this interference level is much greater and also variable, resulting in excessive uncertainty in low level ethanol measurement. Effectively a low level ethanol signal gets ‘buried’ in the noise from the oxygen peak. With a magnetic sector instrument the measurement is very reproducible and methanol and ethanol can be measured with a precision down to 10 ppm.

The standard performance specifications for our Thermo Scientific Prima PRO magnetic sector MS is shown in Table 3. Precision is the standard deviation observed over 24 hours. Note the extremely high precision — 0.05% relative over 24 hours for oxygen. The analysis time including stream switching time is 20 seconds per stream for all 6 components. This reduces to 10 seconds per stream if methanol and ethanol are omitted from the analysis.

Component	Concentration Range	Standard Deviation
<b>Nitrogen</b>	0-100 %mol	0.005 %mol
<b>Oxygen</b>	0-100 %mol	0.005 %mol
<b>Argon</b>	0-1 %mol	0.001 %mol
<b>Carbon Dioxide</b>	0-10 %mol	0.1% relative or 0.0003 %mol**
<b>Methanol</b>	0-1 %mol	2% relative or 0.001 %mol**
<b>Ethanol</b>	0-1 %mol	2% relative or 0.001 %mol**

**Table 3:** Example of standard performance specification for Magnetic Sector MS. \*\*Whichever is greater. Analysis time includes switching time (20 sec/stream for the above 6 components).

## Scale Up from Laboratory to Bulk Production

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 4A shows an example of a mass spectrometer suitable for fermentation process development, the Thermo Scientific Prima BT MS, Figure 4B shows an example of a mass spectrometer suitable for production process monitoring, the Thermo Scientific Prima PRO MS. Both systems share the Thermo Scientific magnetic sector analyzer for high precision multi-component gas analysis and both offer the uniquely reliable Rapid Multistream Sampler.



**Figure 4:** A. Prima BT process development MS. B. Prima PRO process MS.

## Long Term Stability Data

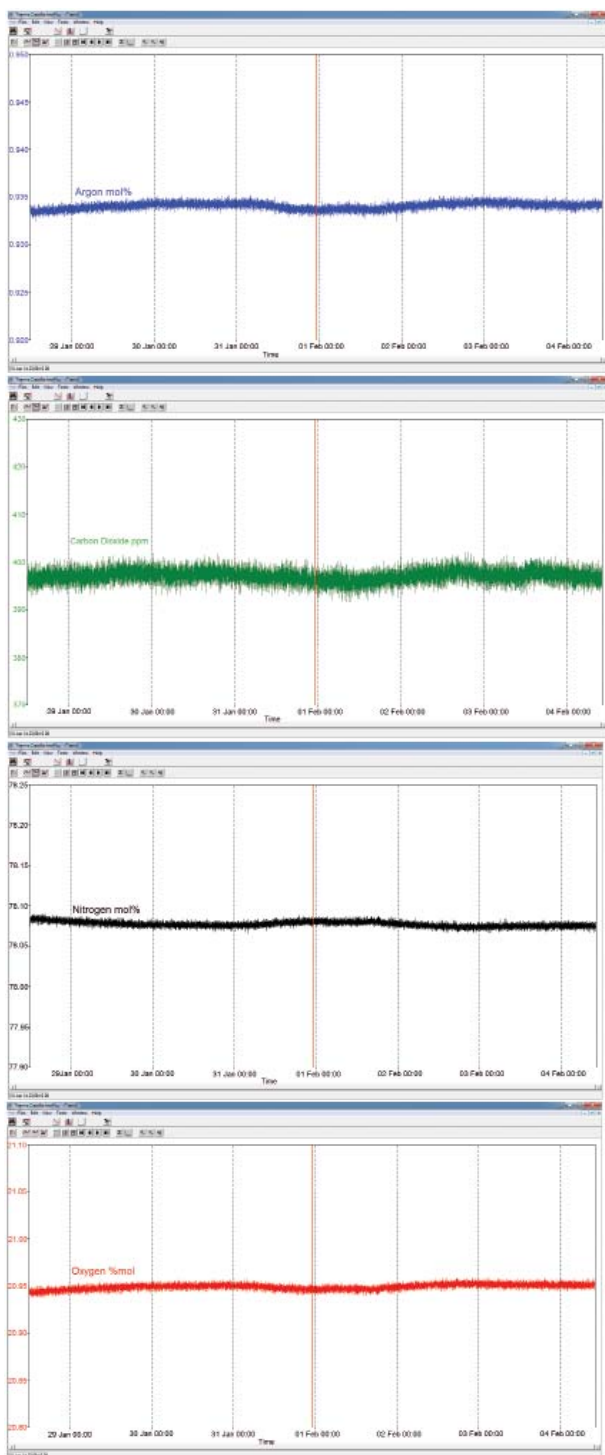
A Prima BT benchtop gas analysis mass spectrometer was configured to analyze Nitrogen, Oxygen, Argon and Carbon Dioxide in a cylinder of compressed air continuously without interruption or recalibration for seven days. The analysis cycle time was 5 seconds to measure these four components. A statistical summary of the results is shown in Table 4 below.

	N <sub>2</sub>	N <sub>2</sub>	O <sub>2</sub>	O <sub>2</sub>	Ar	Ar	CO <sub>2</sub>	CO <sub>2</sub>
Day	%mol Mean	%mol St Dev	%mol Mean	%mol St Dev	%mol Mean	%mol St Dev	ppm Mean	ppm St Dev
1	78.0807	0.0028	20.9459	0.0026	0.9337	0.0003	396.84	1.31
2	78.0767	0.0023	20.9494	0.0023	0.9342	0.0003	397.46	1.25
3	78.0761	0.0024	20.9500	0.0023	0.9342	0.0003	397.34	1.28
4	78.0798	0.0023	20.9469	0.0023	0.9337	0.0003	396.31	1.31
5	78.0777	0.0030	20.9487	0.0028	0.9339	0.0003	396.76	1.34
6	78.0741	0.0023	20.9518	0.0022	0.9344	0.0003	397.47	1.27
7	78.0750	0.0023	20.9512	0.0022	0.9342	0.0003	397.23	1.30

**Table 4:** Example of the Prima BT mass spectrometer’s long term stability data.

Figure 5 shows graphical displays of the four gas readings, taken from the Thermo Scientific GasWorks software’s Data Review Plus module. This long term stability is only available from a magnetic sector MS – quadrupole mass spectrometers require frequent calibration to correct for their inherent drift.





**Figure 5:** Long term stability data from Prima BT mass spectrometer. Argon (% mol), carbon dioxide (ppm), nitrogen (% mol), and oxygen (% mol) (top to bottom) plots from Thermo Scientific GasWorks software.

## Summary

Magnetic sector mass spectrometers have demonstrated the highest levels of precision for fermentation off-gas analysis and have 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 Thermo Scientific Prima BT and Prima PRO mass spectrometers provide fast, precise off-gas analysis through every stage of the fermentation and cell culture processes from laboratory to pilot plant to bulk production.**

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