

Direct Measurement of Respiratory Quotient (RQ) in Fermentation Processes



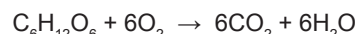
Figure 1: Typical fermentor installation.

ProLine and ProMaxion for Fermentation Off-Gas Analysis

The Importance of Respiratory Quotient

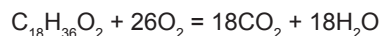
We can understand a great deal about cell metabolism by monitoring the off-gas from fermentors. The gas composition will change depending on the energy source the cell is using. One ratio that is particularly useful for understanding cell metabolism is the Respiratory Quotient (RQ). This can be defined as the ratio of the number of carbon dioxide molecules produced by an organism to the number of oxygen molecules consumed.

RQ is useful because the amount of CO₂ and O₂ produced and consumed depends on which nutrient source is being metabolized. Carbohydrates (general formula C_nH_{2n}O_n) are an important source of nutrition. For example, if we take n = 6 we have the formula for glucose, C₆H₁₂O₆. We can describe the metabolic reaction of glucose by the following equation:



For glucose, RQ is 6 molecules of CO₂ divided by 6 molecules of O₂ and equals one.

If stearic acid replaces glucose, the metabolic reaction becomes:



For stearic acid, RQ is therefore 18 molecules of CO₂ divided by 26 molecules of O₂ and equals 0.7. We can then compare the cell metabolism under different conditions (temperature, pH, agitation, nutrient level, etc.) by simply comparing RQ.

We can convert number of molecules to volume by dividing by Avogadro's number (10¹⁹ molecules / cm³ for standard temperature and pressure):

$$RQ = \frac{CO_2 \text{ molecules}}{O_2 \text{ molecules}} \times \frac{10^{19}}{10^{19}} = \frac{Vc}{Vo}$$

Since the conversion factor appears in numerator and denominator, we can simply use the volume of carbon dioxide released (Vc) divided by the volume of oxygen consumed (Vo) as a substitute for the number of molecules.

Historically dedicated gas analyzers—paramagnetic for oxygen, non-dispersive infra-red (NDIR) for carbon dioxide—have measured the oxygen and carbon dioxide in the air going into the fermentor and the off-gas exiting

Introduction

The biotechnology industry has expanded rapidly over the past five years with growth rates consistently above 15%. The growth has been fuelled by the increasing use in therapeutics of large molecules based on fermentation rather than the more traditional synthesis of small molecules. This has placed greater demands for quality control on all aspects of the product development and production processes, not least on the key stages of cell culture fermentation.

This application note describes the important role that AMETEK mass spectrometers play in the development and production of fermentation products. It should be noted that these are not limited to the pharmaceutical industry – processes as varied as biofuels, biodegradable plastics can benefit from the mass spectrometer's powerful analytical performance.

the fermentor. These analyzers measure concentration in volume percent or volume ppm, so the RQ calculation becomes:

$$RQ = \frac{(\%vol \text{ of } CO_2\text{out} \times FLOW_{out}) - (\%vol \text{ } CO_2\text{in} \times FLOW_{in})}{(\%vol \text{ of } O_2\text{in} \times FLOW_{in}) - (\%vol \text{ } O_2\text{out} \times FLOW_{out})} = \frac{CER}{OUR}$$

CER is defined as the Carbon Dioxide Evolution Rate, OUR as the Oxygen Uptake Rate.

The measurement of flowrates introduces errors that limit the accuracy of the RQ measurement: mass flowmeters are typically only accurate to ± 5%. In addition, the relatively slow analysis speed of paramagnetic and NDIR analyzers limits the number of fermentors that can be monitored with one set of discrete analyzers. This can lead to further errors in RQ measurement if one set of analyzers is used to measure air feed and another to measure off-gas.

Benefits of Mass Spectrometry

Analysis Speed

AMETEK quadrupole mass spectrometers can measure and report the four major air gas components (nitrogen, oxygen, argon and carbon dioxide) in as little as two seconds. Therefore, multiple reactors can be monitored with a single instrument. Even allowing for stream switching and settling times, the analyzer can measure the RQ within 10 to 20 seconds per fermentor. Air feeds and off gases are monitored with one instrument allowing concentration data to be compared and catalogued easily and more accurately.

Wide Dynamic Range

Mass spectrometry's wide dynamic range allows accurate measurement of gas species from ppm to percentage levels. Other techniques require multi-point calibrations to cover the component ranges that are found in the analysis. In some cases they even require different analyzers such as when measuring around 300 ppm carbon dioxide in air feed, or percentage levels of carbon dioxide in the off-gas.

Accurate RQ Calculation without Flow Measurement

The mass spectrometer not only measures the respiratory gases but also nitrogen and argon. We can use this to eliminate the flow measurement. For most standard fermentations, nitrogen is neither consumed nor produced by the process; it follows that:-

$$(\%vol \text{ of } N_2\text{in} \times FLOW_{in}) = (\%vol \text{ } N_2\text{out} \times FLOW_{out})$$

Therefore:

$$FLOW_{out} = \frac{\%vol \text{ of } N_2\text{in} \times FLOW_{in}}{\%vol \text{ } N_2\text{out}}$$

We can now calculate CER and OUR by:-

$$CER = \{CO_2\text{out} \times (N_2\text{in} \times FLOW_{in})/N_2\text{out}\} - (CO_2\text{in} \times FLOW_{in})$$

$$OUR = (O_2\text{in} \times FLOW_{in}) - \{O_2\text{out} \times (N_2\text{in} \times FLOW_{in})/N_2\text{out}\}$$

And RQ becomes (now without flow):

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

Note that although the RQ calculated using nitrogen concentration correction is exactly the same value as that calculated using the classical flow correction method 1, the numerator and denominator are not CER and OUR. They may more correctly be referred to as CDI and OXR, carbon dioxide increase and oxygen reduction.

If the fermentation does actually consume or produce nitrogen (for example, nitrogen fixing micro-organisms), then argon volume concentrations in and out can be used to make the flow correction.

| | Nitrogen | Oxygen | CO ₂ | N ₂ In/N ₂ Out | CDI | OXR | RQ |
|--------------------|----------|--------|-----------------|--------------------------------------|-------|-------|-------|
| Air Inlet | 78.595 | 20.491 | 0.047 | 1.000 | | | |
| Fermentor 1 | 78.700 | 20.193 | 0.255 | 0.999 | 0.207 | 0.326 | 0.637 |
| Fermentor 2 | 78.785 | 20.179 | 0.189 | 0.998 | 0.141 | 0.361 | 0.391 |
| Fermentor 3 | 78.698 | 20.178 | 0.271 | 0.999 | 0.224 | 0.340 | 0.660 |
| Fermentor 4 | 78.776 | 20.178 | 0.194 | 0.998 | 0.146 | 0.360 | 0.407 |

TABLE 1: Example of RQ calculation using the nitrogen concentration correction (actual process MS field data).

Benefits of AMETEK Process Mass Spectrometers

Choice of Analyzer Package

AMETEK offers two analyzers for fermentation off-gas analysis.

► **PROLINE**

Benchtop configuration with up to 16 sample points, for General Purpose locations.

► **PROMAXION**

Process configuration with up to 32 sample points for harsh environment locations.



Both systems use the same AMETEK quadrupole analyzer. With more than 6,000 units installed worldwide, AMETEK mass spectrometers provide performance, reliability and ease of use. The AMETEK product range assists in the scale-up process, from laboratories and pilot plants using ProLine to full production facilities using ProMaxion—the results will correlate from one analyzer to the other.



Fermentation Off-Gas Analysis

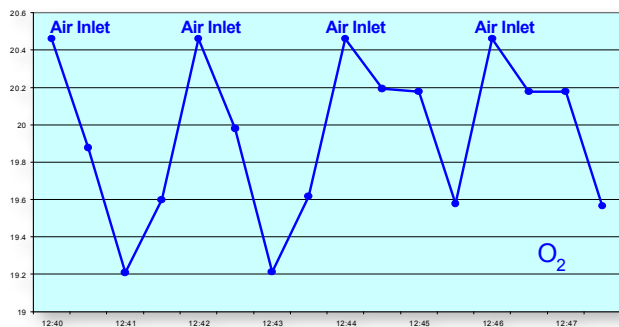
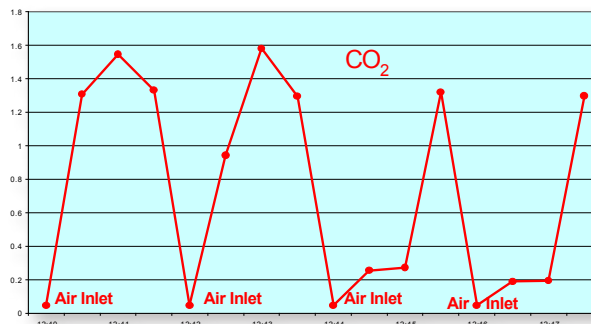


Figure 2. System switching between several fermentors and common air inlet.



Powerful, Flexible Software

AMETEK's Process 2000 software provides automatic control of sample switching and calibration (Figure 2). Analog and histogram scans help to fingerprint fermentations, and trend displays monitor concentrations. Air gases, as well as volatiles such as alcohols, can be monitored down to ppm levels. Autotune algorithms ensure the analyzer is always optimized and the software's powerful calculation facility makes it

easy to set up the RQ calculations. Figure 3 shows an example of how this can be done.

Process 2000 supports industry standard communication protocols like Modbus and OPC for reliable transfer of analytical data to process control systems. And if OUR and CER calculations need to be handled in Process 2000, optional analog input hardware can take flow signals into the OUR/CER calculations.

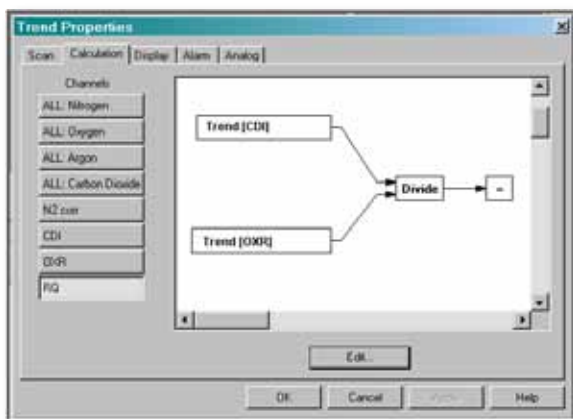


Figure 3. Process 2000 calculation capability.

Summary

AMETEK's ProLine and ProMaxion mass spectrometers provide multipoint, multicomponent analysis of fermentation off-gas. They measure not only respiratory gases, but nitrogen, argon and volatiles as well.

The accurate measurement of nitrogen (or argon) means the systems can calculate and report Respiratory Quotient information with much greater accuracy than traditional methods based on flow measurement. This information can be transferred to process control systems by analog or serial communication protocols.

Optional 21CFR Part 11 Process Software complies with FDA requirements for data security and validation.

As with all AMETEK process analyzers, ProLine and ProMaxion are supported by our international service organization.



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One of a family of innovative process analyzer solutions from AMETEK Process Instruments.
 Specifications subject to change without notice.

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