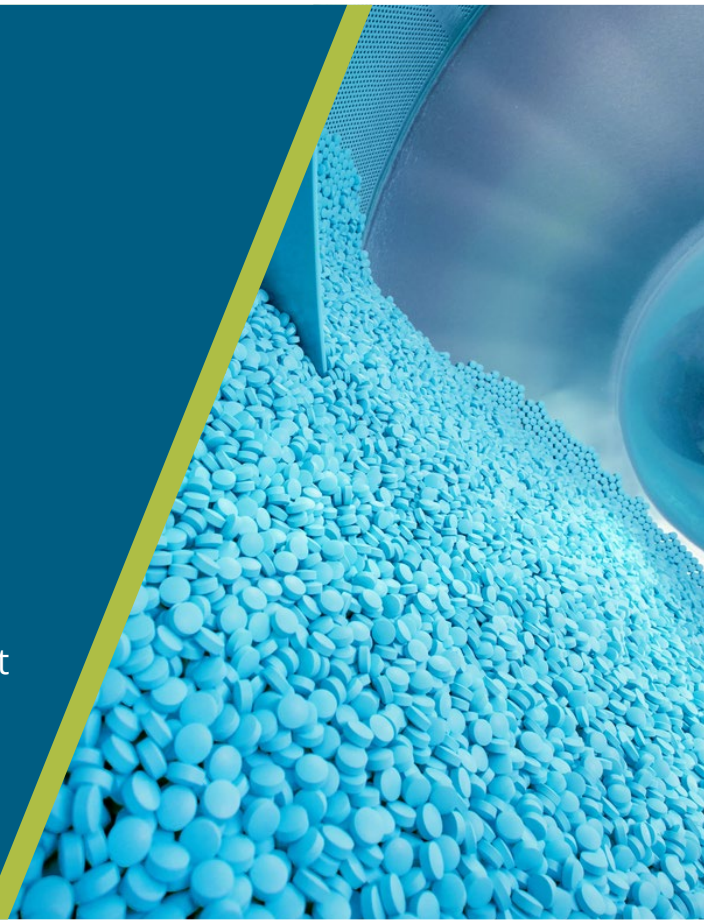
Process Insights™
Premium Insights Into Process

MASS SPECTOMETERS

APPLICATION NOTE



PROCESS AUTOMATION

Fermentation and Cell Culture

Online analysis in pharmaceutical development and production application

Key Words:

Antibiotics, vaccines, prophylactics, hormones, feed supplements, amino acids, industrial enzymes, food additives, vitamins, biomaterials

Strictly speaking the term 'fermentation' actually 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 nutrients, under either aerobic or anaerobic conditions. This definition will be used throughout this application note.

So, 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 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 micro-organisms are referred to as anaerobic.

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 titer (yield).

It is vital that the method used for measuring both sparge gas and reactor effluent is capable of fast, precise analysis. The speed of MS makes it ideal for the fermentation application since a single analyzer can be used to monitor multiple fermentors and bioreactors.

Respiration Metrics

Microbial fermentation is the preferred production method for chemical compounds and therapeutic proteins that don't require post-translational modifications; where a specific shape is required to activate the therapy. These more complex molecules are produced using cultures of mammalian or insect cells rather than micro-organisms such as Escherichia coli or Streptomyces. In all cases, the nutrients provided in the growth medium are consumed in the mitochondria of eukaryotic cells, where the energy molecule ATP is produced in a series of chemical reactions driven by oxygen consumption in the electron transport chain. Figure 1 shows that when carbohydrates are processed, an extra molecule of ATP is produced so the cells go for these nutrients first during the logarithmic growth phase. This is when the microbial population can double every 10 minutes or so. The chemically-defined growth medium provides only enough sugar to achieve a suitable cell density for production. Once this is consumed the cells switch to lipids (fats) or proteins to acquire their energy. This marks the start of the productive phase of the process.

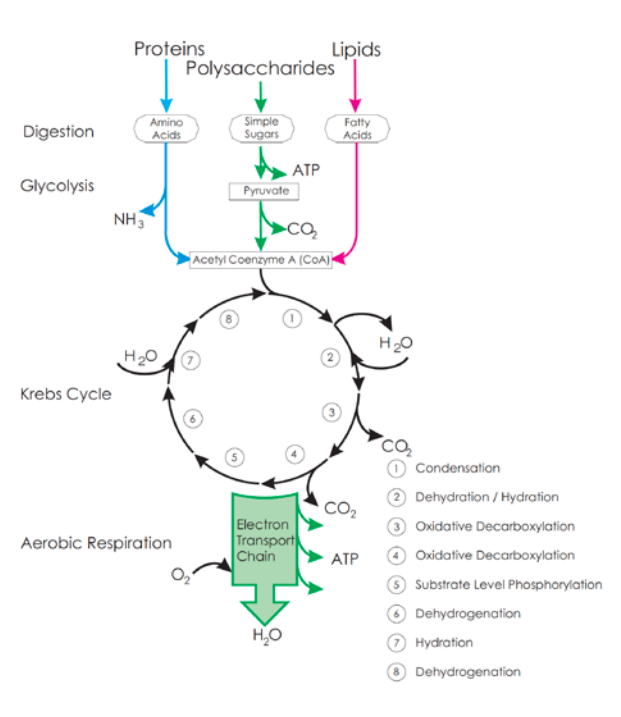


Fig 1. Cellular Respiration

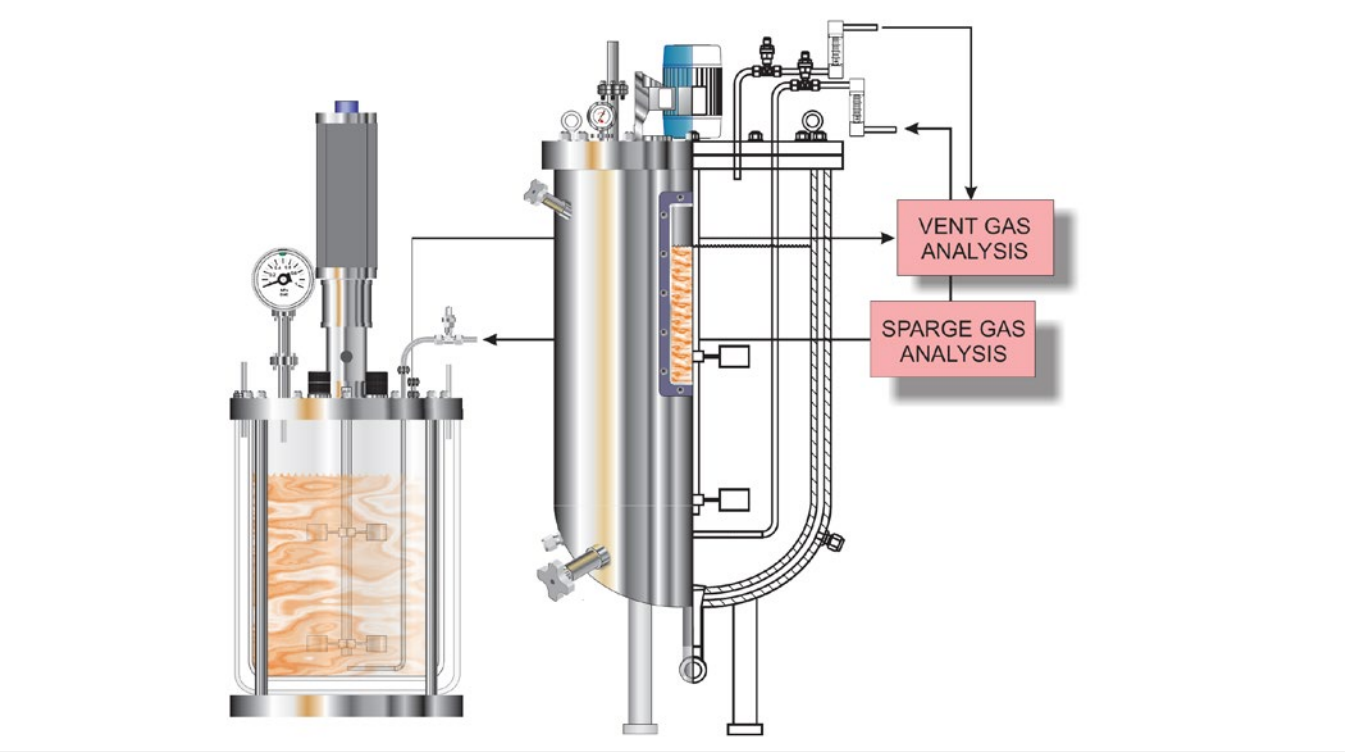


Fig 2. Bench-Top and Pilot Scale Fermentors

Our MGA™ Mass Spectrometer is equipped with a fast and reliable multi-stream selector with a flushing time of <2s. There are four available configurations that provide 16 ports, 32 ports (Figure 3), 50 or 100 ports. The combination of this device and the MGA multi-collector mass spectrometer facilitates a new gas composition measurement every 3 seconds so the effluent from100 fermentors can be analyzed in about five minutes.

The stepper-motor driven stream selector is bi-directional, temperature controlled and includes flow validation.

Fig 3. Phoenix Multi-Stream Sampler

Respiratory Quotient

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) where:

CER = (CO_2 out x flow out) - (CO_2 in x flow in)
OUR = (O_2 in x flow in) - (O_2 out x flow out)

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 mass spectrometer, which can measure N_2 and Ar in addition to O_2 and CO_2. 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 vapour. The calculation for RQ using nitrogen as the flow correction can be expressed as:

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

Figure 4 illustrates a typical plot where the RQ is stable at 1.0 during the early growth phase. After 90 hours or so the RQ value drops, indicating glucose depletion. At this first event the oxygen uptake rate indicates that there is insufficient viable cell density to achieve acceptable titer. At this point additional glucose was added to encourage further cell growth. At the second depletion event, the OUR indicates an acceptable viable cell density so the productive phase is initiated. Without the online analysis providing a course correction, the result would have been a sub-standard titer.

Figure 5A tells a similar story, where the defining event was the depletion of a necessary amino acid (l-Glutamine) which wasn't corrected. 5A shows a traditional offline measurement of total cell density. The growth trajectory looks fairly normal with the exception of a dip 80 hours in.

The plots in 5B show that the OUR took a severe turn indicating cell death. Although the cell density looked acceptable at the end of the batch, the viable cell count was a third of what it would have been if the amine depletion had been detected and corrected.

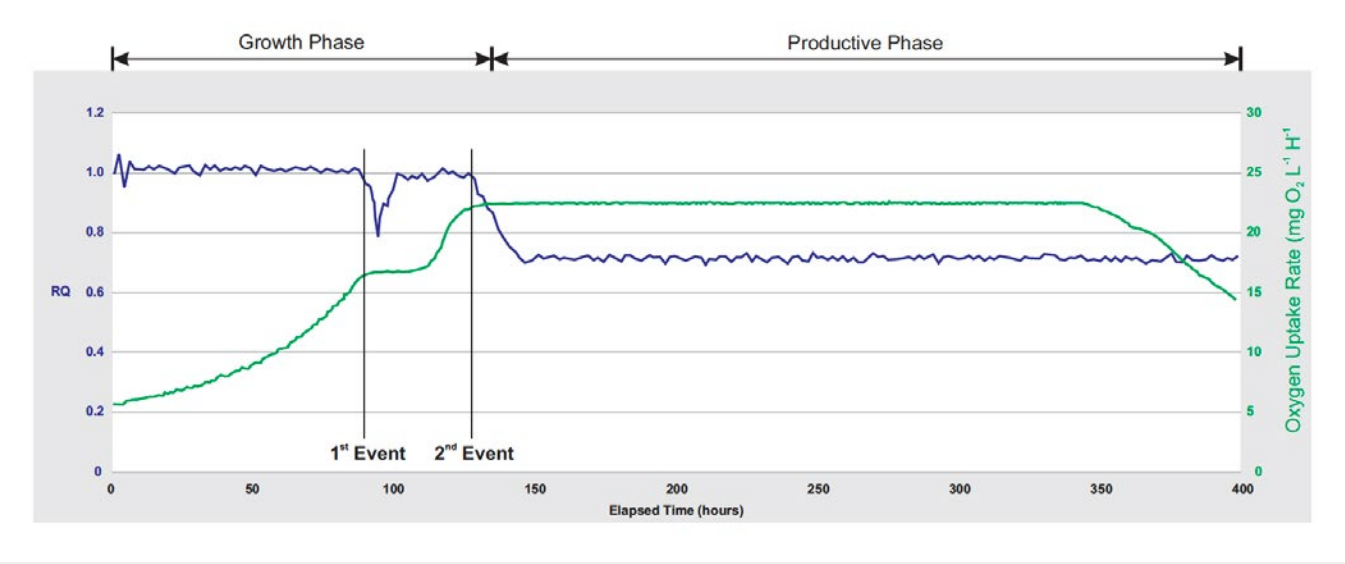


Fig 4. RQ OUR Plot

Hybridoma Culture Growth

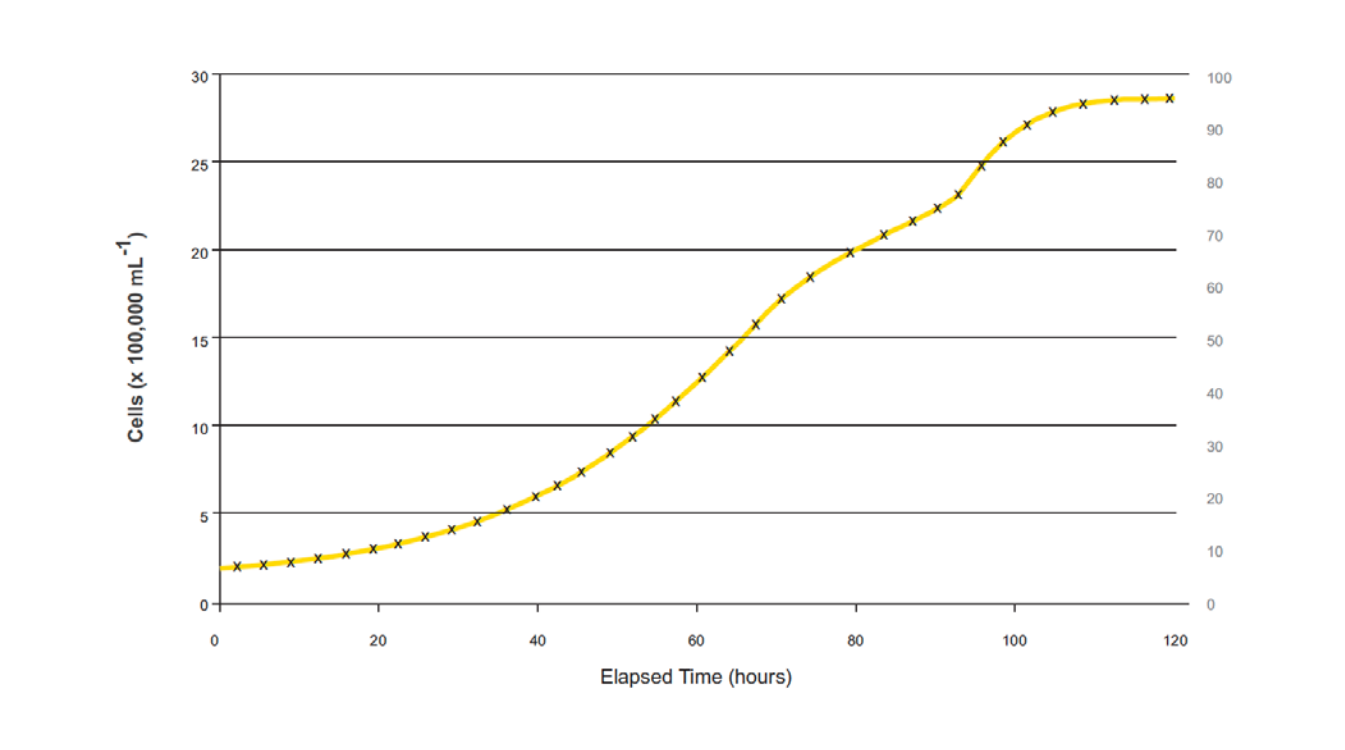


Fig 5A. Offline Total Cells

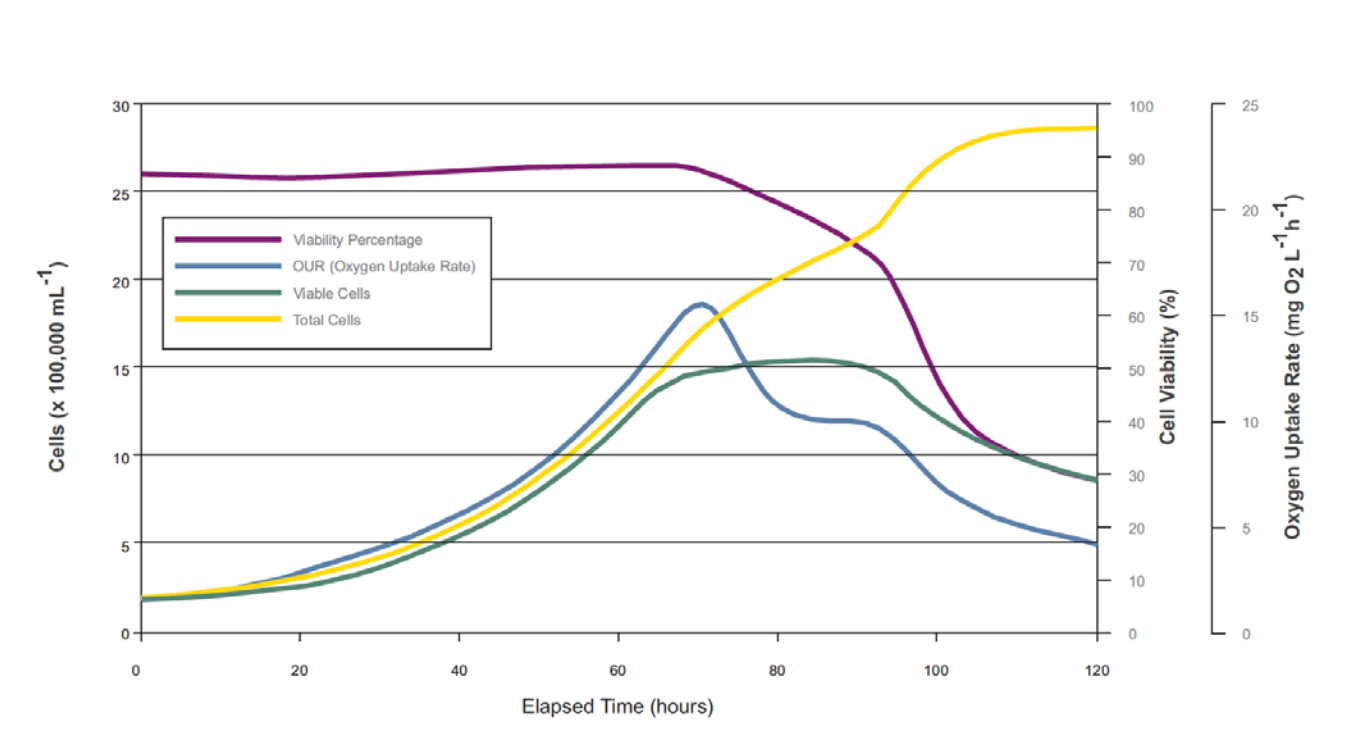
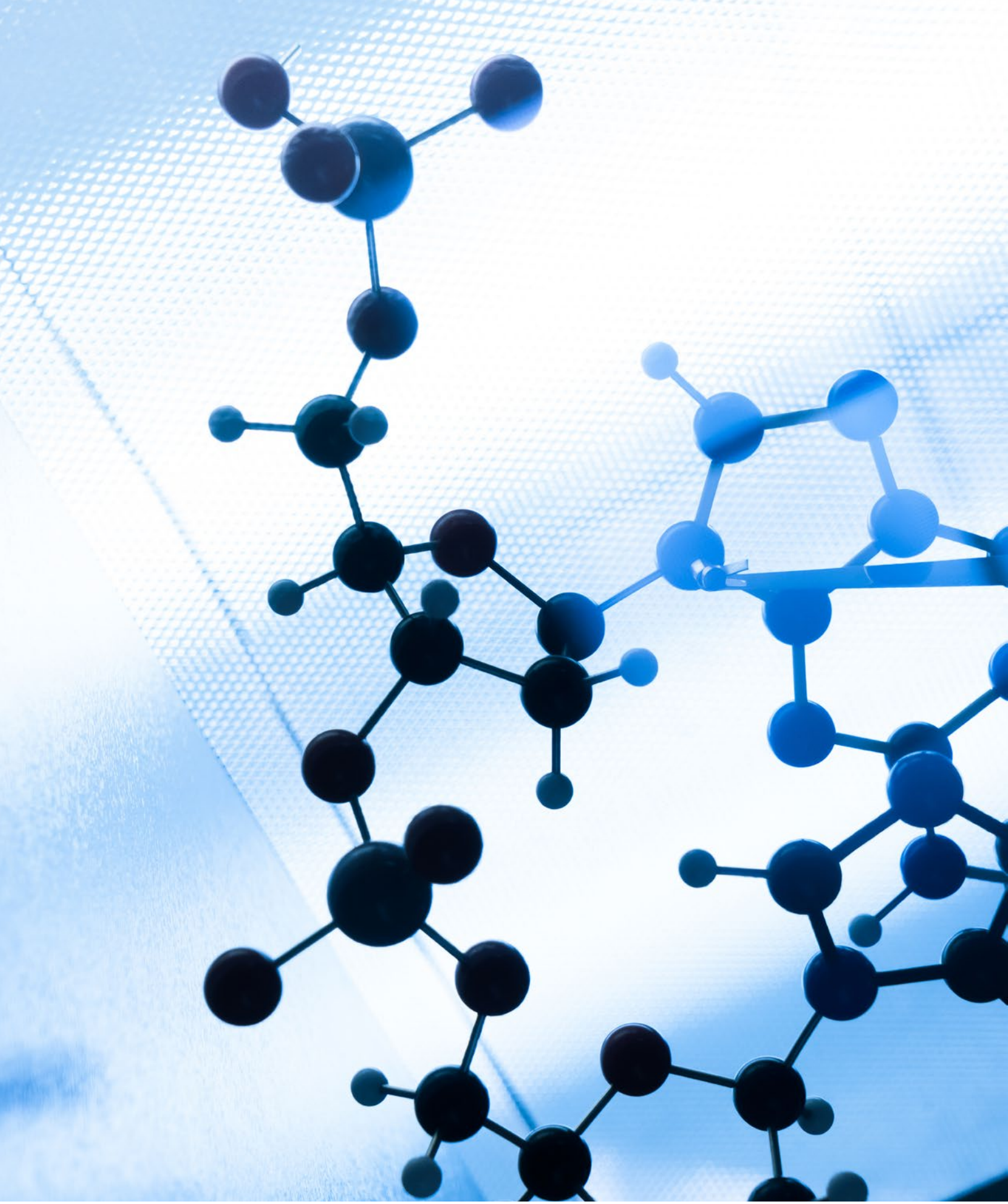


Fig 5B. Online OUR



Sample Conditioning Systems

Fig 6. Standard Fermentation SCS with Membrane Filters and Common Drain Header

Sample Conditioning Systems (SCS) are necessary to ensure a representative sample is delivered to the process mass spectrometer. The panel illustrated above provides protection against liquid carryover and regulates the flow into the multi-stream sampler of the MGA 1200CS™.

The SCS is designed for installation on the right-hand side of the mass spectrometer so the 16 inlets are on the right and the outputs are on the left.

Value Proposition

Mass Spectrometers Provide:

- Best available precision
- Complete gas composition analysis
- Analysis of both sparge and effluent
- Pre-inoculation contamination check
- Accurate OUR, CER and RQ
- Ensure viable cell density
- Improves process understanding during drug development and scale-up – speeds time to market

Process Insights™
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MASS SPECTOMETERS

GAIN REAL-TIME INSIGHT INTO YOUR PROCESS

Process Insights manufactures and delivers premium sensors, monitors, detectors, analyzers, instrumentation, and software that are mission-critical to keep your operations, personnel, and the environment safe – every day across the globe.

Get the most reliable, precision analytical technologies available on the market today. We will work to match your needs and budget, and provide the optimal, and most stable process analysis solution for your application.

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Process Insights is committed to solving our customers' most complex analytical, process, and measurement challenges everyday.

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