Real-time Bioprocess Monitoring of Mammalian Cell Cultures by Magnetic Sector Gas Analysis MS

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ABSTRACT

Our magnetic sector mass spectrometers have been used for off-gas analysis on microbial fermentation processes for over 30 years, with several hundred systems installed. They provide fast, precise information on respiratory activity (Carbon Dioxide Evolution Rate, Oxygen Uptake Rate) and the ratio of these two parameters: the Respiratory Quotient. They are also used to detect contamination by looking for small changes in oxygen and carbon dioxide before culture addition, and monitor volatile grades at trace levels, such as methanol and ethanol and hydrogen sulfide. Our analyzers are being used in both development laboratories and manufacturing plants.

Our mass spectrometers are increasingly being used to characterize mammalian cell cultures, and this poster presents two recent examples of work carried out to characterize different cell culture processes by the Department of Biochemical Engineering, University College London, UK (UCL), and National Institute of Bioprocessing Research & Training (NIBRT).

INTRODUCTION

Analysis of the respiratory gases being fed into and removed from the bioreactor is an ideal way of characterizing a 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.

Many fermentations are characterized by small changes in oxygen and carbon dioxide concentrations at critical phases of the fermentation, for example during the lag phase when the micro-organisms adapt to the nutrients. In microbial fermentations, the feed gas composition is relatively constant – either air or air enriched with oxygen. Mammalian cell cultures are more complex – respiratory activity can be extremely low at key stages in the process, sparge gas conditions tend to be low and sparge gas composition is often changing due to the mixing of several compounds (e.g. nitrogen, oxygen and carbon dioxide). Sparge concentration ranges vary dramatically, for example carbon dioxide can vary from less than a part per million to tens of percent. It is therefore vital that the method used to measure off gas is capable of fast, precise analysis with a very wide linear dynamic range, essential when measuring from low ppm to high %.

Magnetic sector mass spectrometers offer the unique combination of speed, precision, accuracy, long intervals between calibrations and resistance to contamination. Typically, analytical precision is between 2 and 10 times better than the alternative 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 1.

RESEARCH PROJECT 1: UCL

A GS-CHO-K1 cell line expressing a chimeric IgG1 monoclonal antibody was used in a fed batch process in two 5 litre bioreactors. Figure 2 shows the oxygen inlet and outlet profiles measured online using a Thermo Scientific Prima BT magnetic sector MS. Before the Do probe removal, the DO gas profiles contributed by feed and antifoam additions as discussed before are ignored, the DO value, on average, was >1 during the lactate production phase and <1 during the lactate consumption phase.

Figure 3 shows a later run. Respiratory data from the MS had increased understanding of the culture such that the respiratory and VCC profiles are much more alike; the batch duration has also increased dramatically.

Figure 4 demonstrates the ability of the MS to capture physical and biological phenomena in the 5 litre bioreactors. The spikes in the Bio 2 trace between 20 and 40 hours was caused by a clogged sterile filter in the gas line and would have gone unnoticed if not for the real-time measurements from the MS. The filter problem was remedied quickly (after checking on the MS traces) and the culture was able to continue normally.

Addition of antifoam was shown to cause an immediate spike in O2 with a proportionate decrease in CO2 and N2. This supports the hypothesis that antifoam reduces the oxygen mass transfer coefficient (kLa) in the culture and as such, an inlet gas supply with higher proportion of O2 was needed. These O2 spikes were thought to be due to the exponential phase (before ~Day 20) because of the rapid proliferation of cells and their subsequent increase in O2 demand. They were more pronounced in the stationary phase after ~Day 10 where cell density was decreasing. Feed additions were also detected in the O2 traces.

Figure 5 shows a run in 50 L SUB. During the run there were instances when the compressed air supply to the bioreactor was lost and the MS detected the changes in the gas composition. The control system was able to switch to a different blend of gas to supply O2 for the cultures, with pure O2 instead of the usual compressed air supplemented with O2.

Figure 6 shows the correlation between O2 and VCC for 5L and 50L bioreactors. O2 was a raw data measurement that was independent of other measurements like bioreactor volume or gas flow rate. This suggests that O2 could be a reliable online indicator of VCC changes in a cell culture.

Figure 7 shows the relationship between RD and lactate metabolism in the 50L bioreactor. This large step change in the RD profiles contributed by feed and antifoam additions as discussed before are ignored, the RD value, on average, was >1 during the lactate production phase and <1 during the lactate consumption phase.

Figure 8 demonstrates the ability of the MS to capture physical and biological phenomena in the 5 litre bioreactors. The spikes in the Bio 2 trace between 20 and 40 hours was caused by a clogged sterile filter in the gas line and would have gone unnoticed if not for the real-time measurements from the MS. The filter problem was remedied quickly (after checking on the MS traces) and the culture was able to continue normally.

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RESEARCH PROJECT 2: NIBRT

Before starting a research project based on a CHO-K1 cell line, NIBRT first assessed the ability of the MS to monitor bioreactor prior to inoculation and detect subsequent contamination. A 1 L vessel was sterilized and fed with cell culture and a contamination event was simulated deliberately after 200 hours by removing the DO probe, compromising the sterile barrier. Figure 8 shows that Prima BT MS was easily able to detect the bacteria-induced respiratory activity.

Figure 9 also shows respiratory parameters CDC, CITC and the ratio of these, RQ, calculated by Prima BT’s software. Carbonate-induced cell metabolism was observed from RD values close to 1 between 20-100 culture hours. Divergence between CDC and OXK values was observed from 125 culture hours onwards, indicating a metabolic switch producing decreased RD values. Off-line metabolism measurements correlated the RD decrease with depletion of glutamine and glucose, required for mitochondrial energy production, and a switch in lactate metabolism which was consumed rapidly until 300 hours. Cell viability then dropped, confirmed by RD of 0, indicating the absence of respiration.

Impact of process variables on off-gas profiles Prima BT was used to evaluate the implementation of process variables in fed-batch cultures performed at 7L scale involving the same CHO-K1 cell line. Variable gas feed composition aimed to maintain high %DO levels, and neutral pH was applied. Figure 10 shows online Prima BT data combined with offline data: between 87 and 120 culture hours a clear drop in RD from 1.1 to 0.7 indicated a cellular metabolic switch concurring with the depletion of glutamine and glucose, similar to that in the previous batch culture.

CONCLUSIONS

Prima BT magnetic sector MS has been implemented for off-gas characterization in mammalian cell culture bioprocesses, demonstrating significant benefits for real-time process monitoring, including:

• Simultaneous real-time monitoring of cell respiratory activity among multiple cultures, correlating with off-line energy measurements including VCC, viability and metabolite profiles, and process data from in-line sensors.
• Early detection of exhaust filter fouling by measurement of bioreactor inlet and outlet gas flowrates, which could result in undesirable increases in bioreactor system pressure.
• Real-time off-gas MS helps identify process deviations during cell culture & can be used to evaluate batch to batch variation, within predefined specifications, for robust manufacturing (even at different sites).

REFERENCES


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