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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 volatiles at trace levels, such as methanol and ethanol and hydrogen sulfide. Our analyzers are being been 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, Dublin, Ireland (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 exist in equilibrium with 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 flowrates tend to be low and sparge gas composition is a frequently changing mixture of several compounds (e.g. nitrogen, oxygen and carbon dioxide). Sparge concentration ranges vary dramatically, for example carbon dioxide can vary from tens of 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.

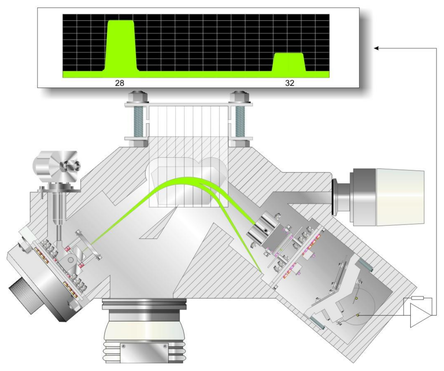


Figure 1. Schematic of magnetic sector MS

RESEARCH PROJECT 1: UCL

A GS-CHO cell line expressing a chimeric Ig4 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 from an early run, with the offline data for Viable Cell Count measured every 24 hours. Although the two bioreactors were controlled by the same control unit, the two profiles are very different. There is significantly greater respiratory activity in Bio 1, which correlates with the higher VCC.

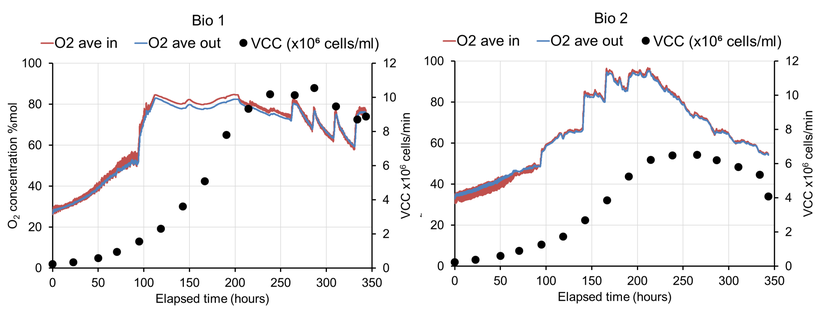


Figure 2. Early UCL experiment producing different O_2 and VCC profiles

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.

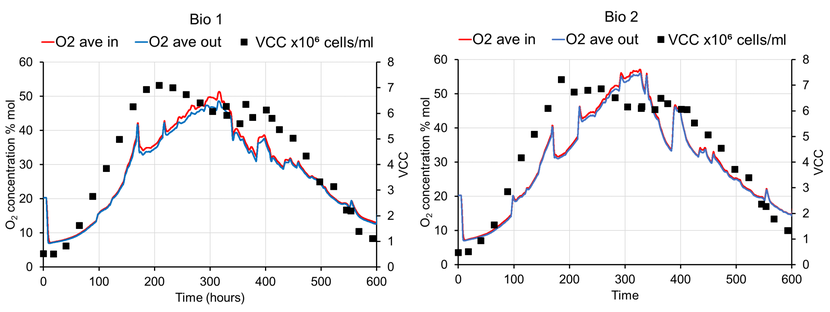


Figure 3. Later UCL experiment producing similar O_2 and VCC profiles

Figure 4 demonstrates the ability of the MS to capture physical and biological phenomena in the 5 litre bioreactors. The spike 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.

Figure 4. MS and VCC data from 5 L runs showing MS ability to identify process issues

Addition of antifoam was shown to cause an immediate spike in O_2 with a proportional decrease in CO_2 and N_2. 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 O_2 was needed. These O_2 spikes were thought to have been masked during the exponential phase (before ~Day 10) because of the rapid proliferation of cells and their subsequent increase in O_2 demand. They were much more pronounced in the stationary phase (after ~Day 10) where cell density was decreasing. Feed additions were also detected in the O_2 traces.

Figure 5 shows a run in a 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 O_2 for the cultures, with pure O_2 instead of the usual compressed air supplemented with O_2.

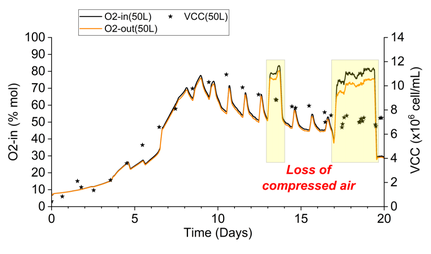


Figure 5. MS and VCC data from 50 L run showing MS ability to identify process issues

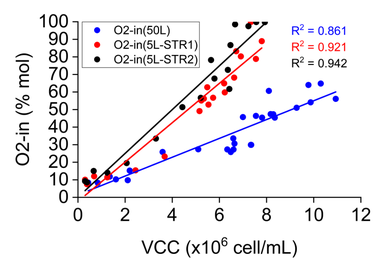


Figure 6. Correlation between O_2in and VCC for 5L and 50L bioreactors

Figure 6 shows the correlation between O_2in and VCC for 5 L and 50 L bioreactors. O_2in was a raw data measurement that was independent of other measurements like bioreactor volume or gas flow rate. This suggests that O_2in could be a reliable online indicator of VCC changes in a cell culture.

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

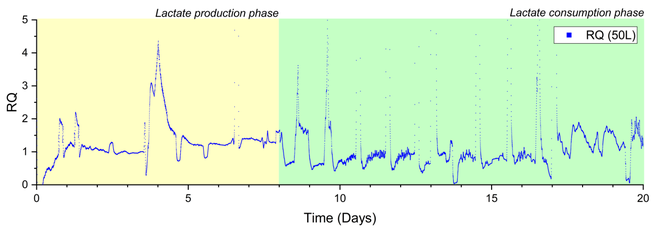
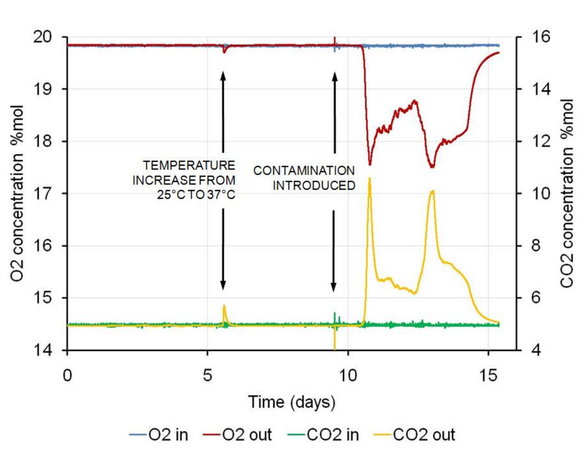
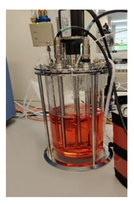


Figure 7. Relationship between RQ and lactate metabolism in 50 L bioreactor

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 sterility prior to inoculation and detect subsequent contamination. A 7 L vessel was sterilized and filled 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.





Before DO probe removal

After DO probe removal

Figure 8. Bioreactor contamination test, showing bacterial respiration

Real-time monitoring of CHO cell culture processes

A batch process was set up using CHO-K1 cell line producing humanized IgG1 antibody. Stable gas feed was provided using air and CO_2, without O_2 supplementation. Figure 9 shows correlation of real-time off-gas profiles with process parameters and performance indicators obtained by daily off-line analysis.

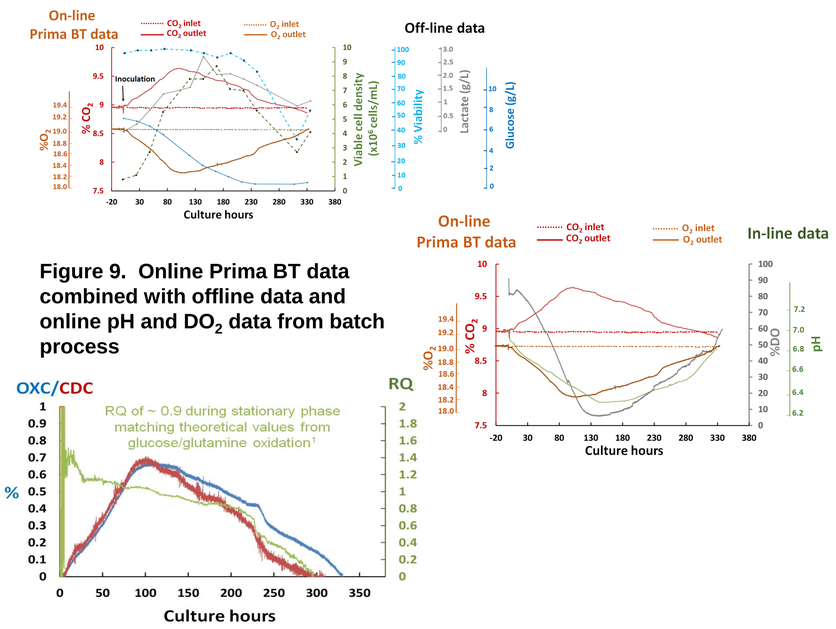


Figure 9. Online Prima BT data combined with offline data and online pH and DO_2 data from batch process

Figure 9 also shows respiratory parameters CDC, OXC and the ratio of these, RQ, calculated by Prima BT's software. Carbohydrate-induced cell metabolism was observed from RQ values close to 1 between 25-100 culture hours. Divergence between CDC and OXC values was observed from 125 culture hours onwards, indicating a metabolic switch producing decreased RQ values. Off-line metabolite measurements correlated the RQ 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 RQ 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 RQ 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

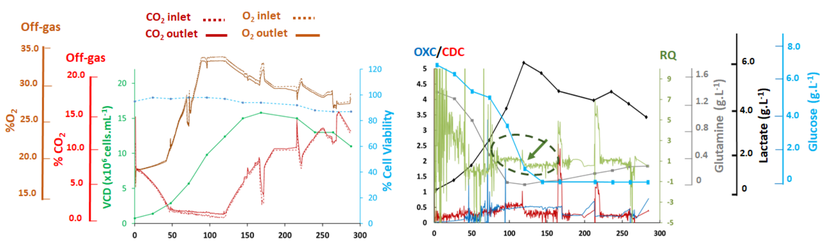


Figure 10. Online MS data combined with offline data from fed-batch process

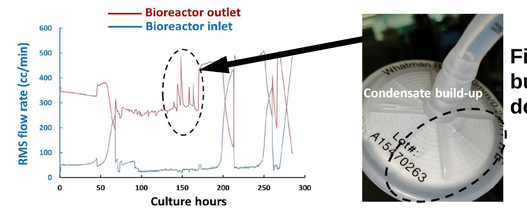


Figure 11. Condensate build-up in exhaust filter detected by Prima BT

Valuable process information was obtained by monitoring the flow-rate of the inlet and off-gas streams as measured by Prima BT. The build-up of condensate in sterile filters can result in filter fouling and an undesirable rise in pressure within the vessel. Discrepancies between inlet and outlet profiles detected by Prima BT were able to highlight the presence of blockages in the filters, as shown in Figure 11.

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 bioreactors, correlating with off-line 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

1. Fitzpatrick L, Jenkins H A & Butler M, Appl Biochem Biotechnol 43: 93-116

ACKNOWLEDGEMENTS

Patrick Floris, Jonathan Bones, NIBRT, Ireland
Hai Yuan Goh, Mike Sulu, UCL UK
Graham Lewis, Consultant, UK

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