Rapid process monitoring & control in mammalian cell culture using off-gas mass spectrometry analysis

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1. **Demonstrate the applicability of off-gas mass spectrometry (MS) analysis in mammalian cell culture (5L glass STR & 50L SUB).**
2. **Determine the implications of various actions or events during cell culture on the MS traces.**

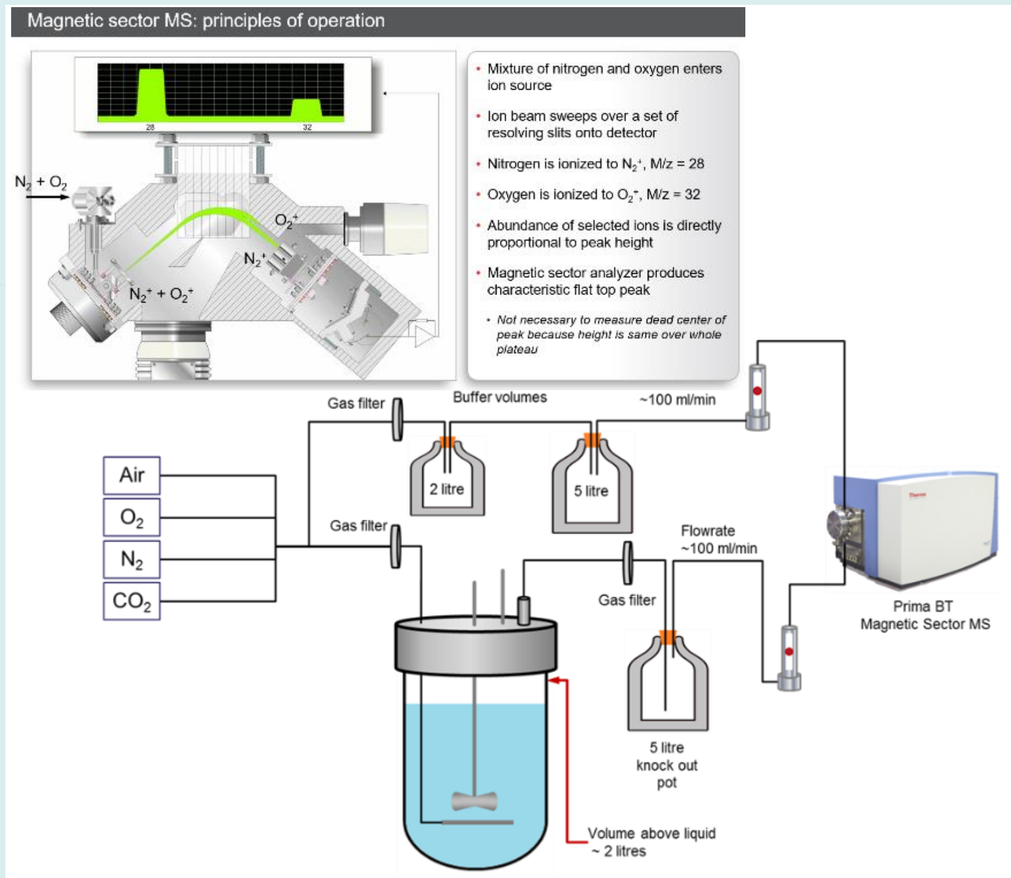
Materials & Methods

- **GS-CHO cell line; Ig4 monoclonal antibody (mAb) cB72.3**

- **5 L glass bioreactor; initial working volume of 2.5 L and inoculated at 0.3 x 10^6 cell/ml**
- **37º C; pH 7.2 via carbon dioxide (CO2) gas and addition of base (1 M NaOH)**
- **Dissolved oxygen at 30 %; horse shoe sparger; single three blade impeller at 45º pitch**
- **Addition of 1% (v/v) antifoam solution**

- **Fed-batch mode; daily sampling; daily bolus feed was initiated when [glucose] ≈ 3g/L**
- **Basal media → CD CHO**
- **Feed media → CHO CD EfficientFeed™ B Liquid Nutrient Supplement**

- **Cell concentration and viability → Vi-CELL™ XR Cell Viability Analyser**
- **Nutrients and metabolites → BioProfile FLEX Analyzer**
- **mAb quantification → 1 mL HiTrap Protein G column, Agilent 1200 HPLC**
- **Off-gas analysis (gas streams in & out of the bioreactor) → Thermo Scientific Prima BT Mass Spectrometer**



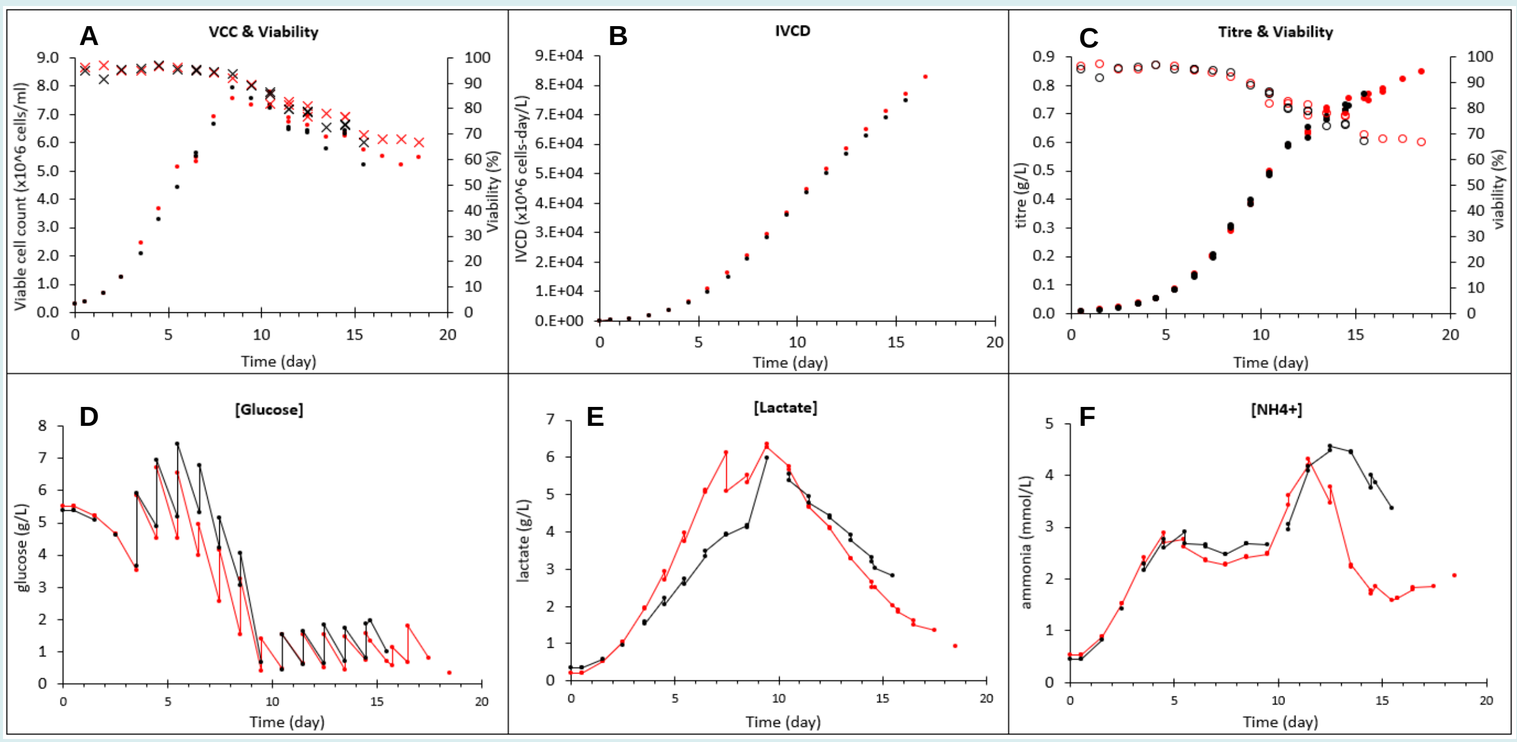


Figure 1. Overlay of the various offline data of the duplicate 5L runs, (A) Viable cell concentration & Viability, (B) Integral viable cell density, (C) Titre & Viability, (D) Glucose concentration, (E) Lactate concentration and (F) Ammonium concentration. Bioreactor 1 & 2 are represented by the colour red & black respectively in each plot.

Results & Discussion

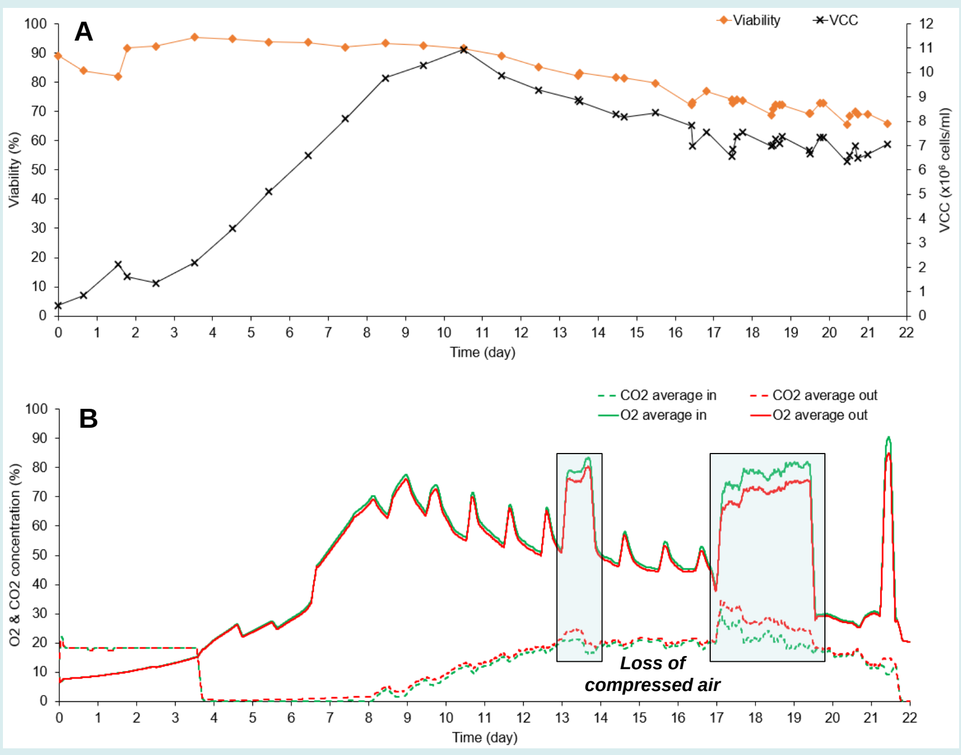


Figure 4. Various offline data & MS gas traces of the 50L SUB, (A) Viability & viable cell concentration, (B) O2-in & -out and CO2-in & -out for 50L SUB.

The MS traces in the 50L SUB are expected and comparable to what have been observed in the 5L runs. This demonstrates the technical feasibility of implementing the off-gas MS technique in a pilot 50L scale as well as benchtop 5L scale, regardless whether it's a glass/stainless steel bioreactor or a single-use bioreactor.

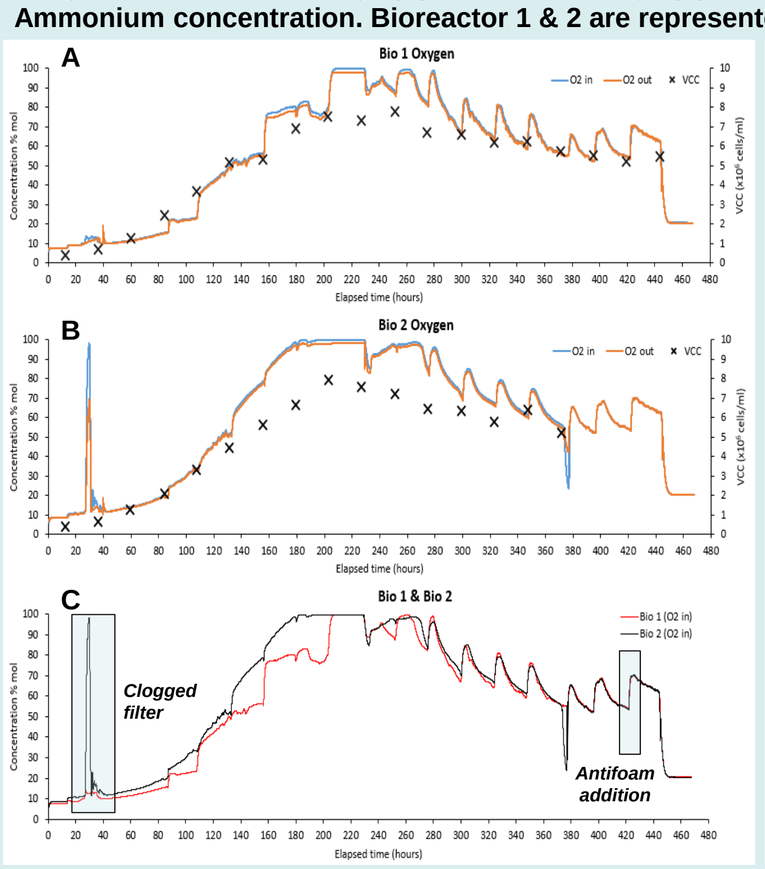


Figure 2. MS O2 gas traces of the duplicate 5L runs, (A) O2-in & -out for Bioreactor 1, (B) O2-in & -out for Bioreactor 2 and (C) Overlay of O2-in of Bioreactor 1&2.

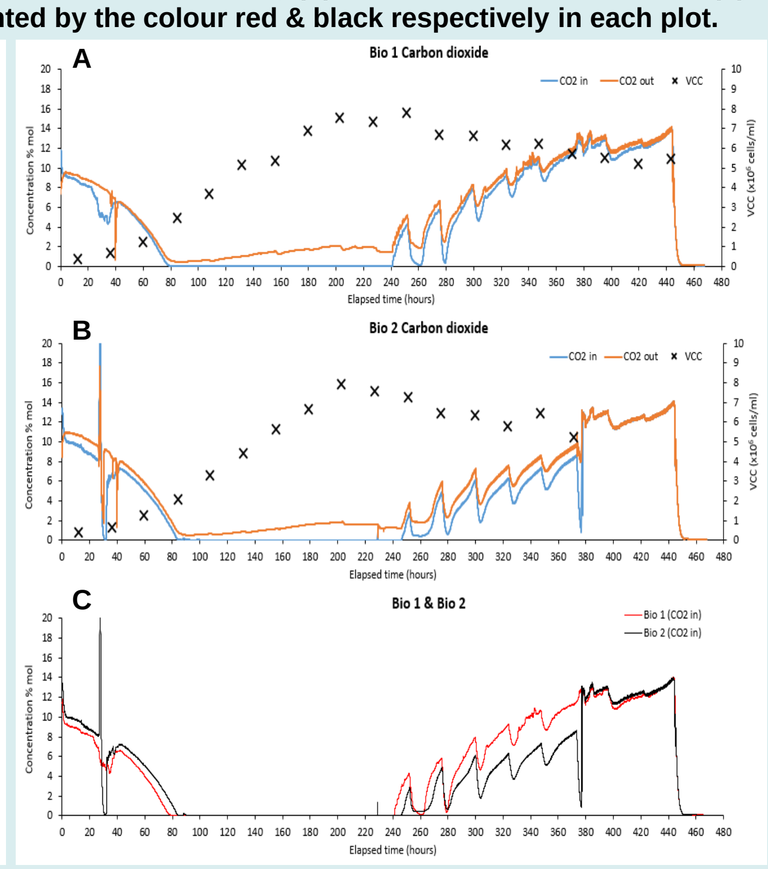


Figure 3. MS CO2 gas traces of the duplicate 5L runs, (A) CO2-in & -out for Bioreactor 1, (B) CO2-in & -out for Bioreactor 2 and (C) Overlay of CO2-in of Bioreactor 1&2.

The MS gas traces are behaving as expected; O2-in is higher than O2-out & CO2-in is lower than CO2-out due to the aerobic respiration of the cells. The reverse can happen at the very beginning of the culture when the cell number is too low or when the measurements of the inlet & outlet gases are out-of-synchronisation.

By taking a comprehensive look at both the offline data & the on-line or real-time measurements from the MS, we have shown that the 2 bioreactor runs are indeed replicates of one another. An added benefit of the MS is the real-time monitoring for process deviations (relating to gases). A spike in the MS trace between 20-40 hours was caused by a clogged sterile filter in the gas line and would in fact have gone unnoticed if not for the real-time measurements from the MS. The problem was remedied quickly (after checking on the MS traces) and the culture was continued as per normal.

Actions / Events	Observations on MS traces
Routine sampling	*No observable effect.*
Clogged sterile exhaust/venting filter	*Disturbance to gas traces observed; magnitude depends on extent of the clog.*
Antifoam addition	*Disturbance to gas traces observed,*
Feed media addition, bolus (<10°C)	*Disturbance to gas traces observed.*
Feed media addition, bolus (temp. same as bioreactor)*	*Disturbance to gas traces observed; comparable to feeding at low temperature.*
Loss of gas	*Disturbance to gas traces observed.*
Glucose addition*	*Non-direct effect; metabolism change results in changes in gas demands/outputs.*

**data not shown*

Factors affecting MS gas traces

- Antifoam.
- Feed temperature is not an issue (at the tested temp. & volumes).
- Any gas perturbation caused by external factors (non-culture related; gas supply leak/loss or blockage).
- Metabolic changes (lactate production vs. consumption).
- Surfactants[#] present in the media.

[#] *difficult to differentiate the effects of glucose and surfactants in the media.*

Acknowledgements

Assistance during the bioreactor setup & the actual runs from Haneen Alosert was appreciated. Schematics of MS setup were provided by Graham Lewis.

Several actions during cell culture can influence the MS traces. The implementation of real-time, off-gas MS in mammalian cell culture can help identify process deviations during bioreactor runs & can be used as a convenient way to evaluate batch to batch variation, within predefined specifications, for robust manufacturing.