

## Introduction

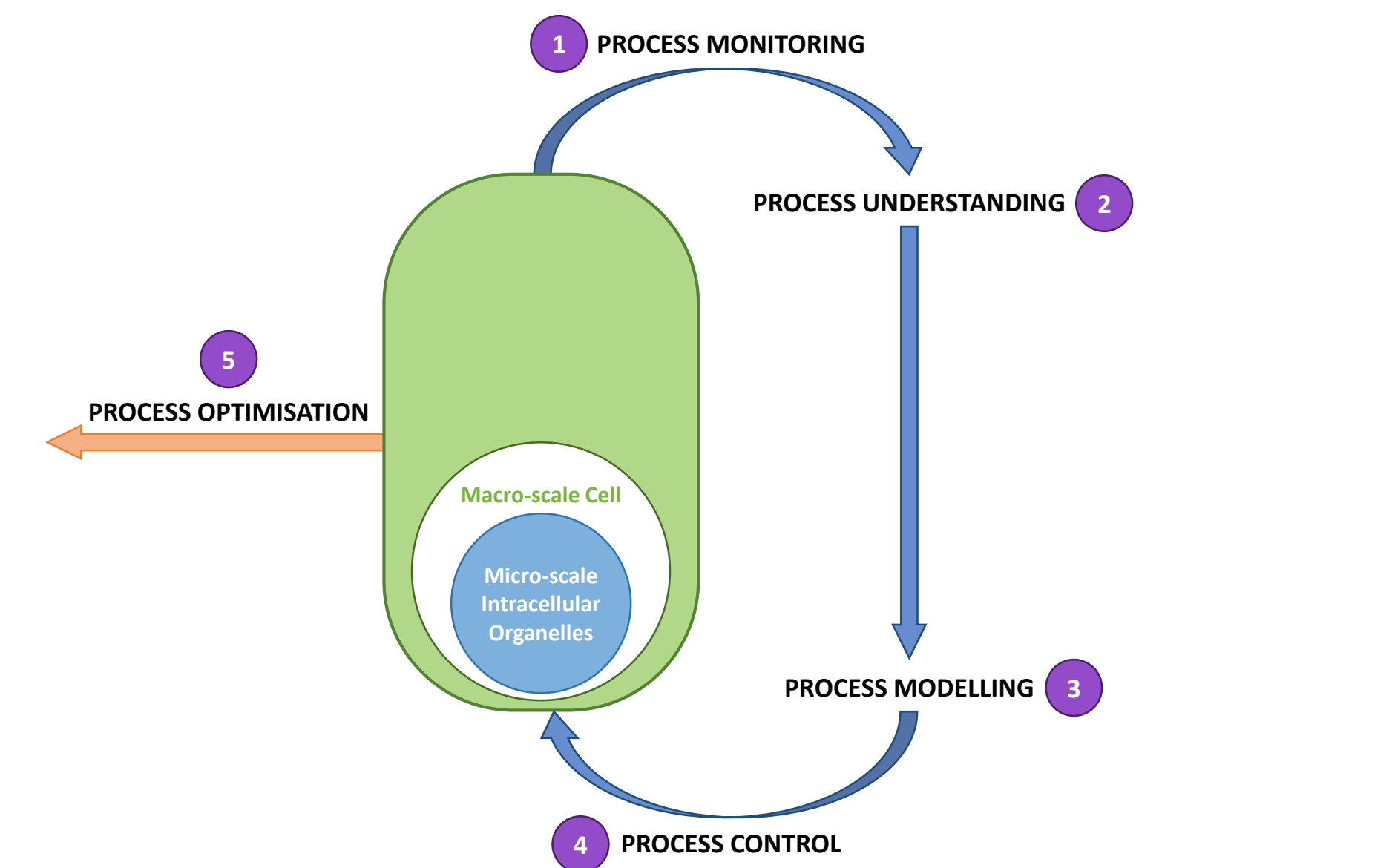
Chinese Hamster Ovary cells are the most widely-used platform for the production of monoclonal antibodies (mAbs) by the biopharmaceutical sector. mAbs are high cost and high value product which typically exceed 1000 €/g and annual sales of over €1 billion. CHO cell mAb manufacturing processes require refinement to ensure that the highest product yield is achieved while ensuring quality. The use of Process Analytical Technology (PAT), such as Raman spectroscopy<sup>1</sup>, enables in-depth interrogation of cell culture operations in order to define optimal feeding and control strategies which reduce product time-to-market and, ultimately, curb drug development costs. This work presents a novel controlled feeding strategy – enabled by real-time Raman analysis – that improves CHO cell growth and productivity by reducing ammonia secretion, a metabolic by-product that has been widely shown to negatively impact cell culture performance and product quality attributes<sup>2</sup>. The work highlights how advanced PAT strategies can be leveraged to gain in-depth process understanding to maximise mAb productivity.

## Summary of approach

- The cell line was initially studied to understand the growth profile and the metabolism of the cells. Amino acid analysis by GC showed media components that were being depleted by the cells, this information was used to define the feeding strategy of the amino acids.
- Critical process parameters were identified using multi variate data analysis (MVDA) through the use of principle component analysis along the batch trajectory.
- Data was gathered using the Kaiser Raman RXN2 along with offline metabolite and cell density data. This data was used to build models using partial least squares regression (PLS) that would allow online interpretation of the Raman signal to quantitative values.
- These values were then used to inform the process through the use of feedback loops. In this case the glucose concentration was controlled at a setpoint.

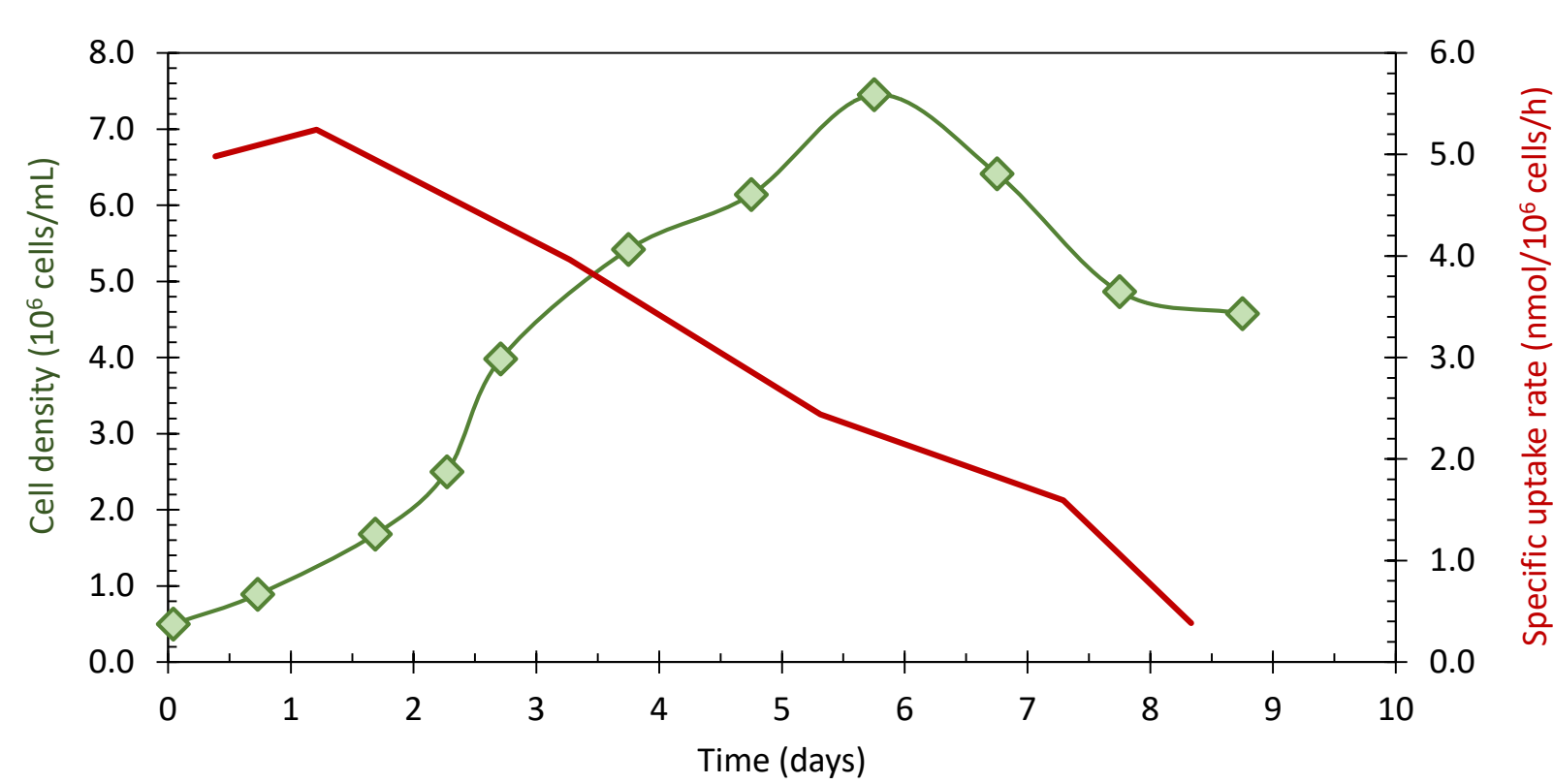
## Experimental

- A CHO-DP12 cell line from ATCC producing an IgG product was studied to enhance a basic bolus fed production process.
- Eppendorf's Dasgip stirred tank bioreactor system was used using 3L glass vessels.
- Metabolite analysis was performed using the Roche bioanalyser and cell count using Roche Hi-Res.
- Amino acid analysis was performed using Phenomenex Ezfaast kit for sample preparation and an Agilent GC with FID.
- Camo Unscrambler X was used to perform MVDA analysis of all data. The batch modelling plugin was used to plot the batch trajectory. Unscrambler X was used to perform partial least squares (PLS) modelling on the offline data with the Raman spectral data.
- Camo Process Pulse was used to implement the PLS models to interpret the Raman spectral data. This data was then fed back to the Dasgip control software to implement a feedback loop to control feeding. Glucose, amino acid and glutamine feeding rates were controlled in these experiments. The glucose concentration was controlled at a set point of 0.5 g/L.



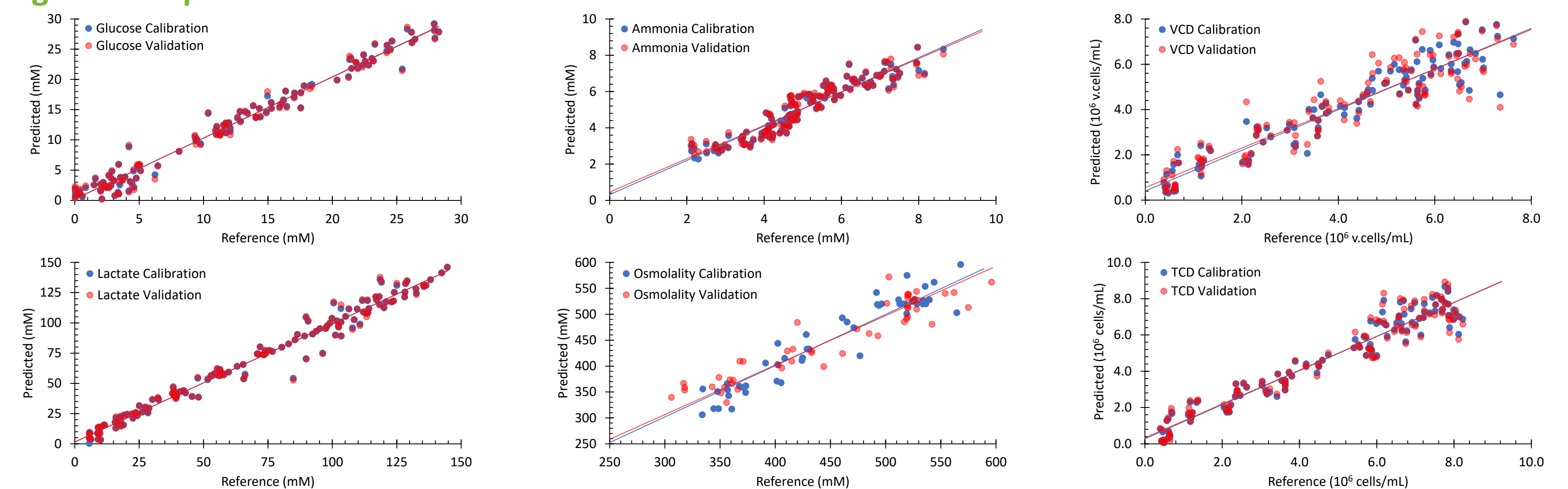
**Fig. 1: Overview**  
 1. The process is monitored through Raman spectroscopy, metabolite analysis, osmolality, pH and DO tracking.  
 2. Understanding of this data is through data analysis by means of metabolite consumption profiles and multivariate data analysis.  
 3. Process modelling by examining Raman profiles along with other in-process parameters.  
 4. Process control is implemented by feeding back metabolite and density data to the controller.

## Amino Acid consumption rates



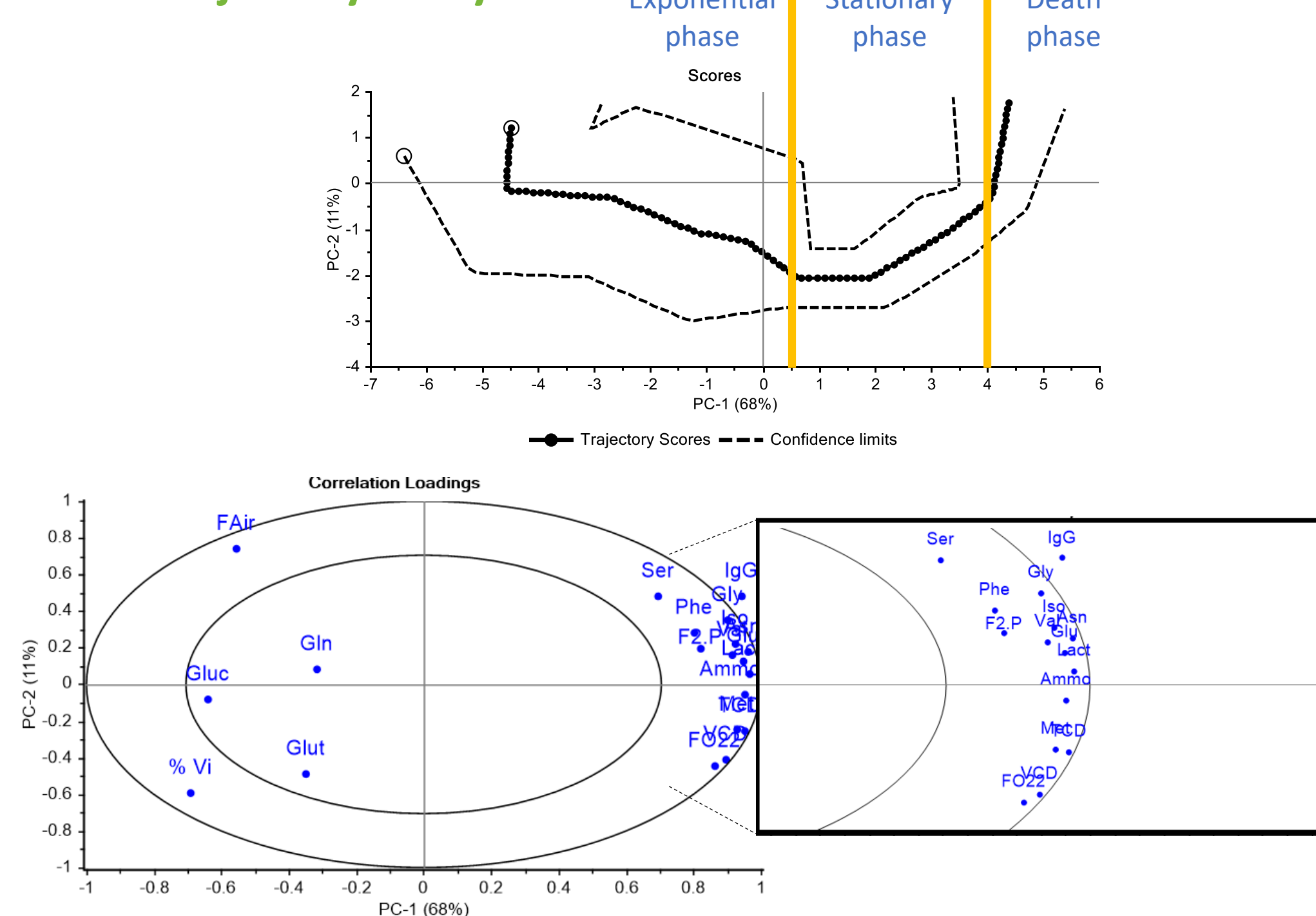
**Fig. 2: Amino acid consumption profiles**  
 Amino acids were examined by comparing their specific consumption/production rates with the growth curve. This information helped to design the feeding strategy and understand their role in growth and production.

## Modelling Raman spectra



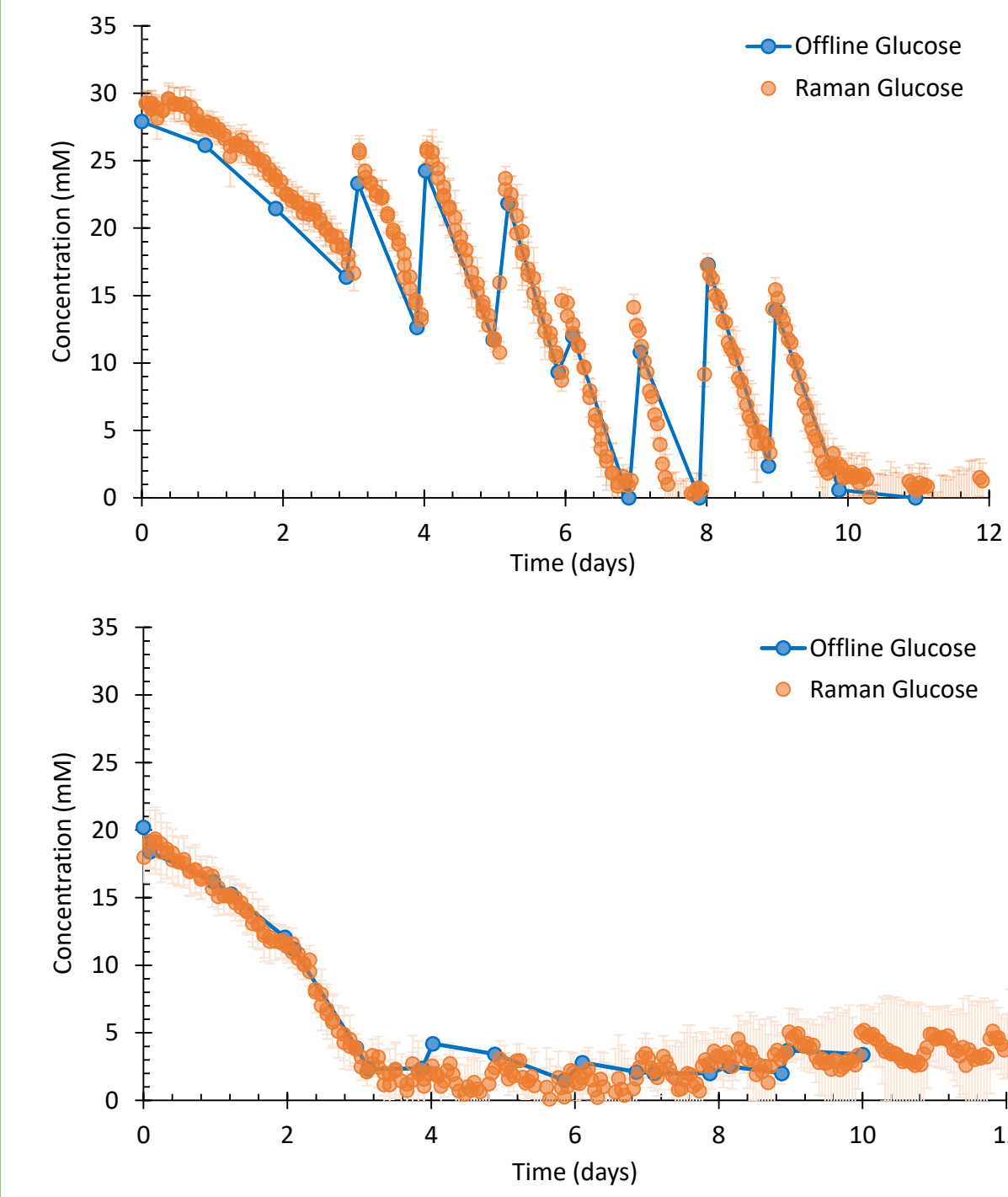
**Fig. 3: Raman models**  
 Offline data used to build Raman models for Total Cell Density, Viable Cell Density, Glucose, Lactate, Ammonia, Osmolality. PLS modelling of Raman data provides strong quantitative correlations with metabolite concentrations.

## Batch trajectory analysis



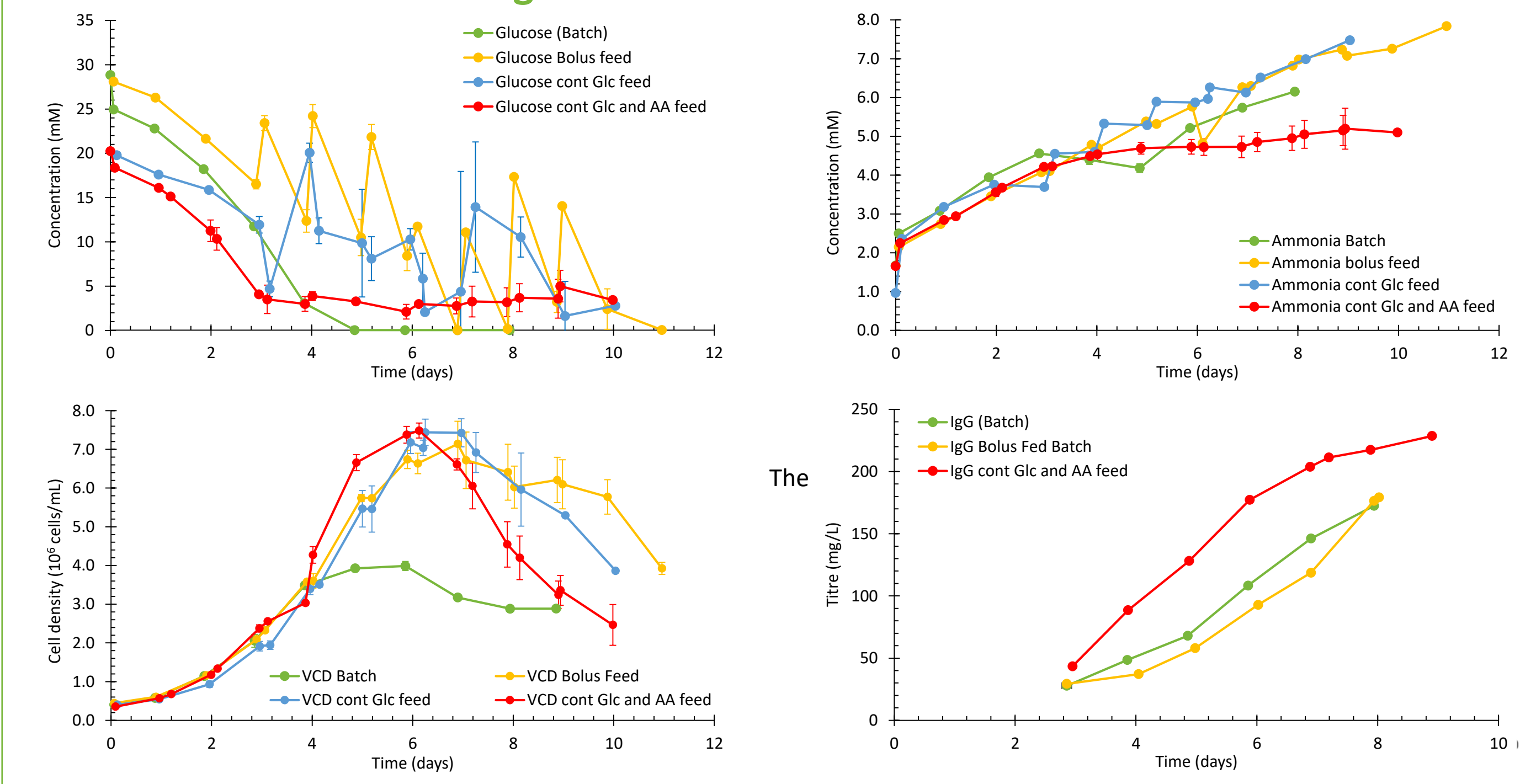
**Fig. 4: Batch trajectory analysis**  
 Above the trajectory of the fed batch results are mapped out. The centre line represents the trajectory of the batch and the outer lines show the confidence limits of the process. The direction of the trajectory represents the loadings at that part of the process. Notice that principle component 1 represents 68% of the variation in the data. The loadings plot below is used to interpret variable correlations for each part of the trajectory.

## Raman controlled feeding



**Fig. 5: Offline and Raman glucose data**  
 Bolus glucose feeding on top and continuous glucose feeding on the bottom show how Raman is used to control feeding

## Result of controlled feeding



**Fig. 6: Comparison of glucose feeding strategies and viable cell density (VCD) profiles**  
 In the figure above notice the progression of glucose feeding control. And below, in yellow notice the sharper growth of VCD when both glucose and amino acid feed were continuously maintained at a low level.

**Fig. 7: Comparison of ammonia profiles and titre profiles**  
 In the figure above notice in yellow that the ammonia profile shows a decreased rate of production. The titre profile shows an increase in growth by comparison to the batch and bolus runs.

## Conclusions

- Gaining a full understanding of the batch process through metabolite analysis and batch trajectory analysis gives further insight into the critical parameters of the process.
- Asparagine, glycine, serine, glutamine and ammonia are important once growth transitions from exponential to stationary phase.
- Batch trajectory analysis is useful for determining which parameters are not important, the variables that align with the important principle components are then investigated to see which are influential and which are merely a correlation.
- Setting tighter limits on these parameters can ensure a better process with more consistent results.
- Feed strategies can improve productivity and reduce the accumulation of harmful by-products such as ammonia, lactate and overall osmolality.
- Bolus feeding leads to exposing cells to widely oscillating nutrient concentrations which may lead to overflow metabolism and therefore suboptimal productivity. See Fig 5 for controlled glucose feeding.
- Through a more controlled feeding of amino acids, the glutamine volume fed was reduced by 60% and the final ammonia concentration was reduced by 50%, see Fig 7 (a). This led to an overall titre increase from bolus fed batch process giving 176 µg/mL to the continuous fed batch giving 217 µg/mL, a 23% increase in titre, see Fig 7 (b).
- Continuously feeding to maintain relatively constant low nutrient concentrations avoids metabolic overflow and results in increased mAb productivity and lower NH<sub>4</sub> concentrations.

## Future work

- The process can be further optimised by defining feeding strategies based on the specific consumption rates of individual nutrients during exponential and stationary growth phases. On-line control could be implemented by determining a minimum specific growth rate, obtained from Raman measurements, which would serve as the threshold to switch from one growth phase feeding strategy to the next.
- The same strategy can be implemented on a process with a different cell line, perfusion processes, alternative media and feed types.

## References

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- Wong et al 2004. Impact of Dynamic Online Fed-Batch Strategies on Metabolism, Productivity and N-Glycosylation Quality in CHO Cell Cultures. Biotechnology and Bioengineering Wiley. DOI: 10.1002/bit.20317
- Kaiser 2013. Bioprocess protocol pre-batch setup guide.

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