Non-invasive MIR Spectroscopy for Glucose Control and Monitoring during Fed Batch Phase

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Introduction

Glucose as the primary carbon source is a critical parameter in mammalian cell culture processes. Typically, glucose is fed as a daily bolus addition leading to a strong fluctuation in glucose concentration during the process. Since the glucose concentration has shown an influence on antibody production, cell growth and glycosylation, a continuous glucose control may improve product quality and quantity [1]. However, a significantly better glucose control requires a much higher frequency of taking and analyzing offline samples and more bolus additions, which leads to increased workload and costs. A non-invasive and real-time process analytical technology (PAT) would solve this issue. Spectroscopic methods, for example, are promising, as they don't require any time consuming sample preparation. However, spectroscopic methods are not widely spread among the biopharma industry [2], due to their relatively high cost and complex calibration procedure. Here we present our mid-infrared (MIR) spectroscopy system "Monipa", which can keep the glucose concentration constant in the fed batch phase using a relative measurement method. By this approach, we eliminate the need of building a complex calibration model, which is time-consuming and requires an extensive knowledge of spectroscopy.

Material & Methods

For online measurement we used our IRUBIS Universal crystal in combination with a single use flow-cell. Monipa can be connected directly to the bioreactor via a loop (see Fig. 1) or can be integrated in the perfusion stream. The first step to control glucose is its reliable measurement.

To evaluate if a relative measurement is feasible, a perfusion run was performed with CHO cells, which was monitored online with Monipa for 12 days. For online measurement the flow-cell was integrated into the perfusion line, between the cell retention device and the permeate bag. The reference sample was taken from a port between cell retention device and flow cell and measured with a Cedex Bio HT device.

The glucose concentration was calculated in real time from the spectral raw data with a linear algorithm which doesn't depend on additional calibration data.

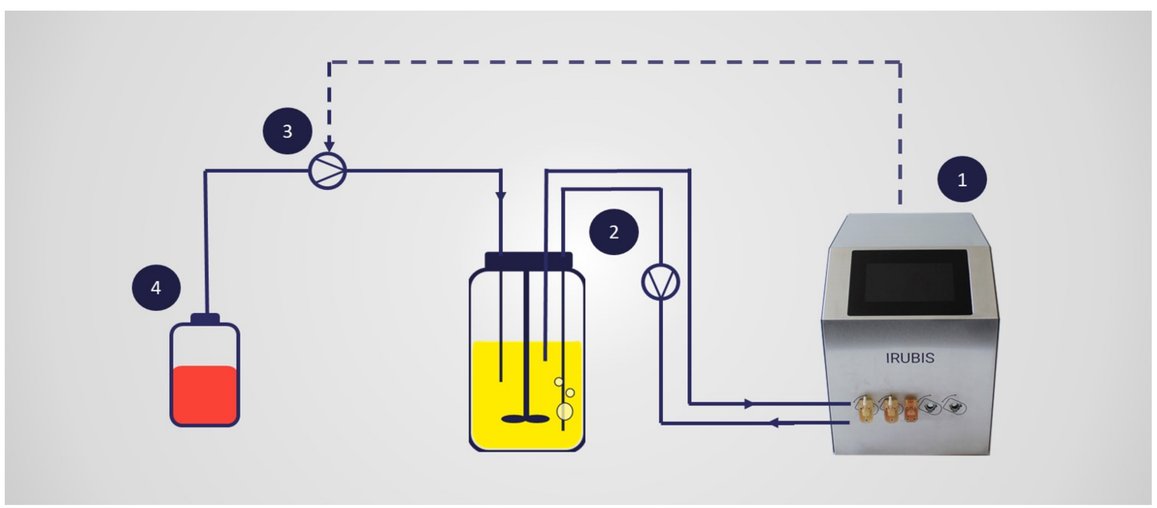

Fig. 1: *Schematic illustration of the used setup. 1) MIR spectroscopy system Monipa, 2) closed loop of bioreactor and Monipa, 3) control loop to feed pump, 4) glucose.*

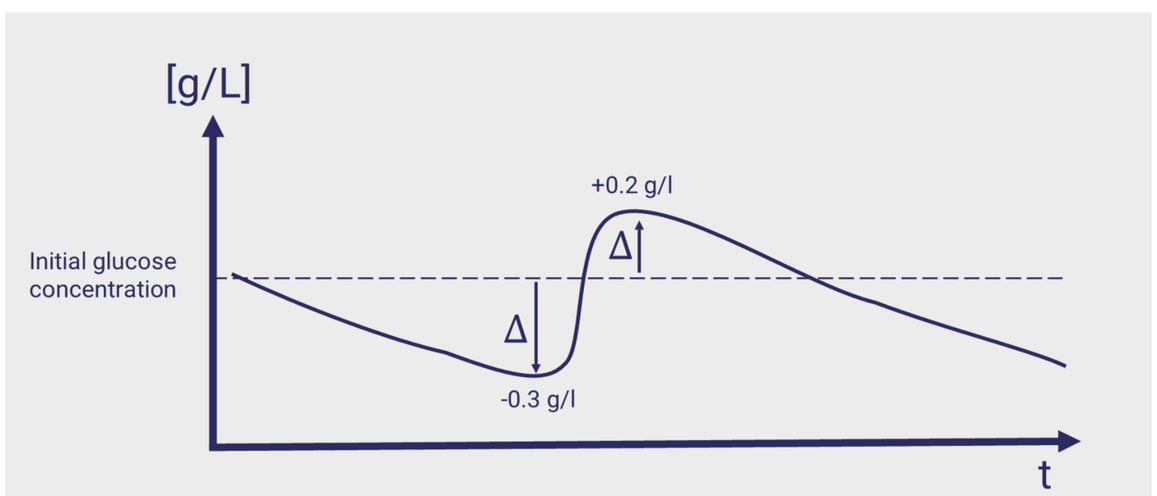

Fig. 2: *Relative measusrement: The glucose level is kept at the initial glucose level by adding glucose.*

Results and Discussion

All offline data was plotted against the online data at the corresponding point of time. The correlation plot shows good agreement with the reference method. The RMSE of 0.17 g/L is comparable to results from literature where an extensive calibration model has been used [2].

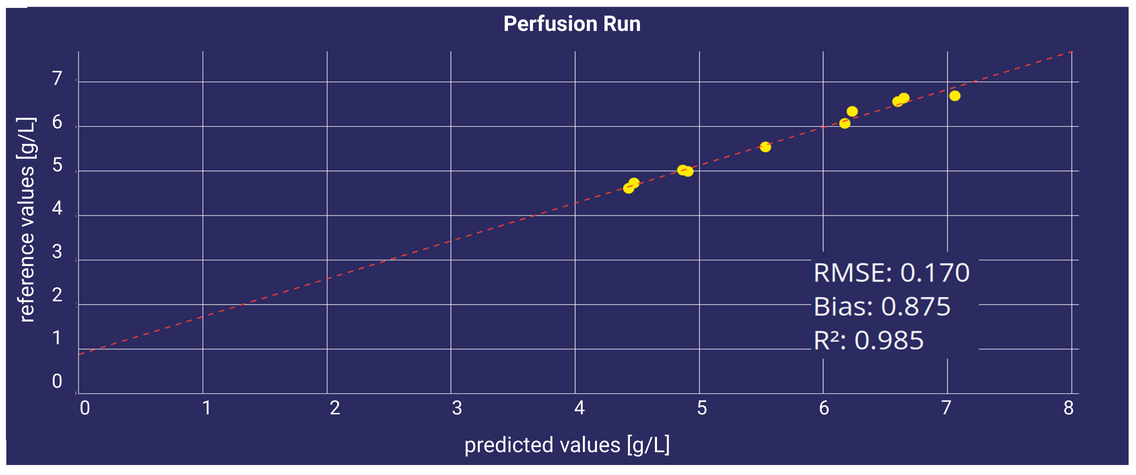

Fig. 3: *Evaluation of prediction accuracy of the relative measurement from a perfusion process in comparison to the reference method.*

However, the bias of 0.875 g/L is an indicator, that our algorithm can be improved to get closer to an ideal bias of 0 g/L. The R^2 value of 0.985 proves the very good correlation between predicted values and reference values.

We would like to point out that the correct synchronization between reference and predicted data is not trivial, because the sampling process by itself interfered with the online measurement. Drawing an offline sample from the tube causes the a physical averaging of the glucose concentration, which is equivalent to a numerical averaging of the online measurement over the same time span. It also confuses the chronological sequence for a short time interval, because the offline sample was removed form the perfusion stream before the online measurement was performed.

Conclusion and Outlook

The relative measurement showed very promising results with a lot of potential for further improvement. The offline and online samples require even better synchronization, to improve the significance of the comparison between offline and online data. The shown results can be seen as an encouragement to work on the next step towards a calibration less glucose control.

The correct PID parameters for the feed pump need to be approximated. Therefore, a water/glucose model can be used, where the initial glucose concentration can be diluted with VE-water to simulate a glucose consumption by the CHO cells.

The control algorithm will try to keep the glucose predictions close to its initial value. The upcoming experiments in the up coming months will be used to implement an fully automated plug&play glucose control with minimal user interaction.

References

[1] Y. Fan, Biotechnol. Bioeng., 2015, 112.

[2] M. Sandor, J. Biotechnol. 2013, 148.

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