Automated sampling and sample processing in CHO processes for process monitoring and media development

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MOTIVATION

Mammalian cells, such as CHO cells, are complicated factories for valuable products such as antibodies or vaccines. **Timely monitoring** and **control** are important tools in order to facilitate **process robustness** and **product quality**. An adequate and accepted reference tool for measurement of product, substrates, media components or metabolites are HPLC methods, facilitating physiological analysis of a process. Usually, sampling and sample processing is done manually, hence, sample analysis is done off-line at the end of the process. In this study, we want to show how this end-point measurements can be avoided as well as the **benefits of automated and timely monitoring of processes**.

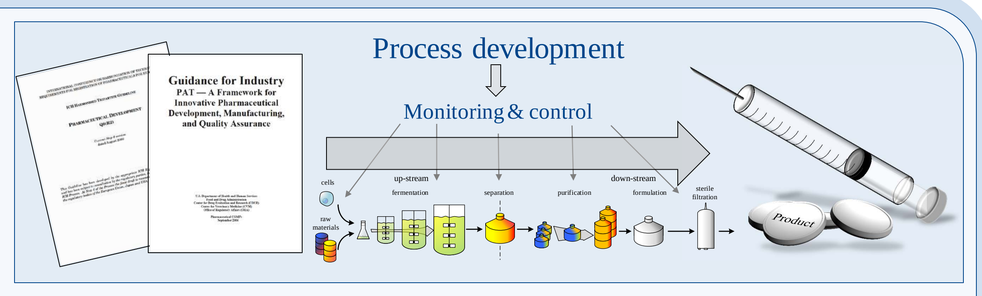


Figure 1: Monitoring via automated sampling leads to availability of data in a timely manner. Hence, it can be used for control actions or facilitates faster decisions in development and production.

SET - UP

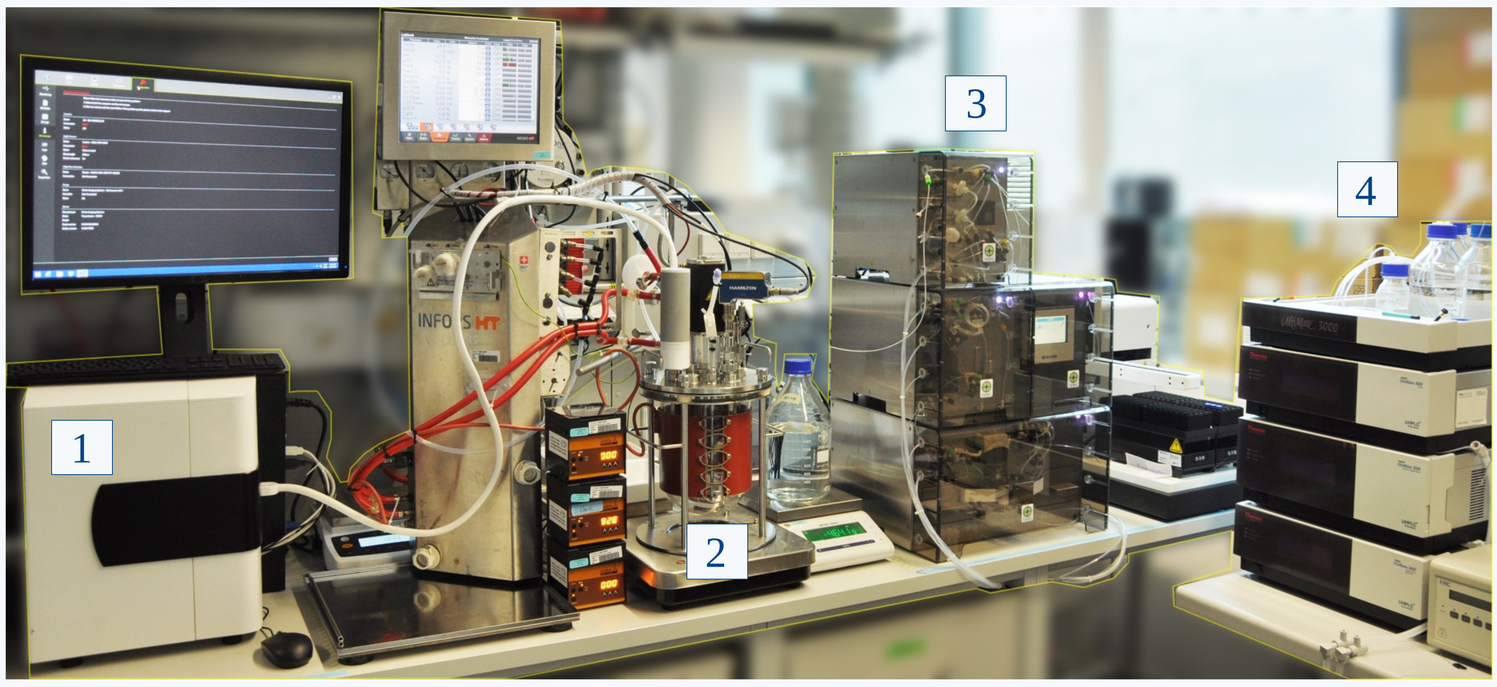
1) iLineF (Ovizio) online microscope
analysis of TCC, VCC and viability every 30 minutes

2) 3.6L bioreactor
control of pH, pO2, pCO2 and T

3) Automated sampling system (Numera®, Securecell)
2.8mL sample is taken from bioreactor, diluted if necessary and filtered → supernatant is stored in vials at 4° C
Sampling is possible every 15 minutes

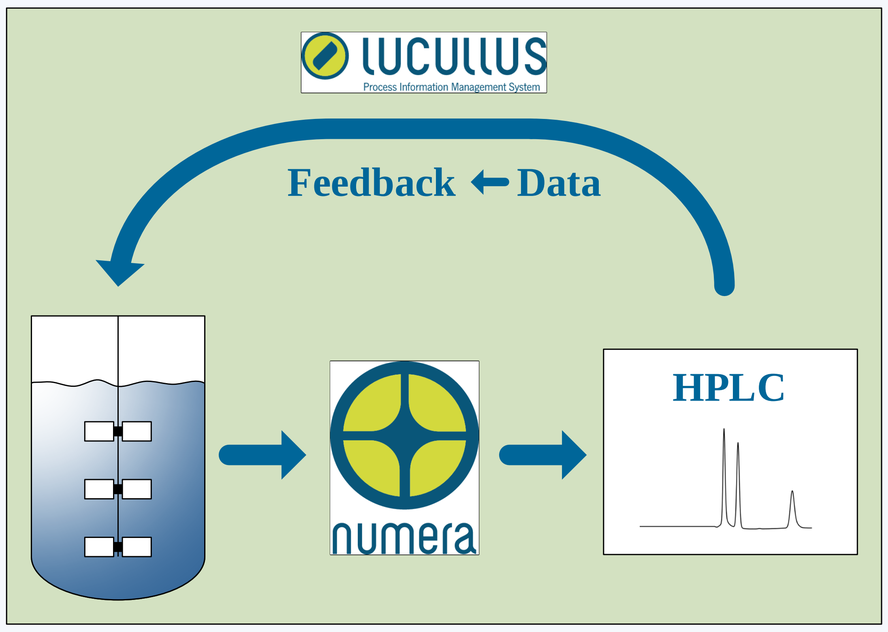
4) HPLC (Ultimate 3000, Thermo)
Enabler of multiple online measurements, e.g. substrates, metabolites or product

5) Software
Chromeleon® → peak integration, calibration
Lucullus → PIMS for data management and control actions



RESULTS

I) Automated sampling enables monitoring



Workflow: Numera and HPLC

- automated sampling every hour
- filtration (0.45µm)
- automatic injection into HPLC
- automated peak integration by Chromeleon®
- calculated value send back to process control system (Lucullus)

→ facilitates monitoring

→ availability of data in a timely manner without manual interaction

→ Possibility of feedback to process (e.g. feeding strategy, setpoint adaptation, process end point decision ...)

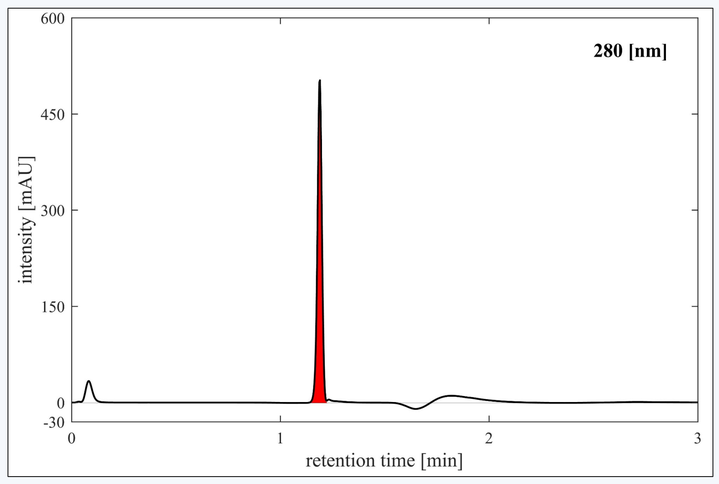


Figure 2: Analysis of product in 3 minutes via HPLC. The product peak is automatically integrated and the concertation is send to the process control system.

II) Real-time product measurement

Advantages:

- Process transparency
- Control actions can be made
- Errors of manual sampling can be recduced
- Faster detection of process end-point (decline in productivity)

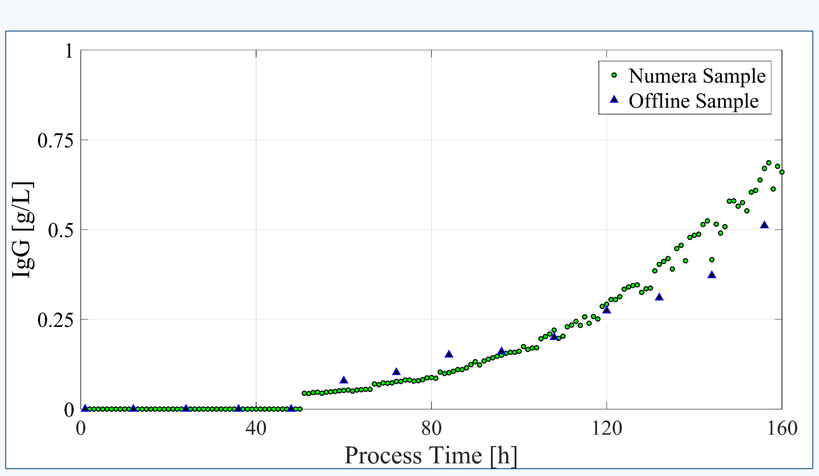


Figure 3: Time course of the concentration of product in the supernatant. Automated sampling (green dots) was performed every hour and manual sampling (blue triangles) every 12h.

III) Media development – real-time analysis of media components

Advantages:

- Reduction of time and cost
- Faster evaluation of limiting or inhibiting substances possible
- Data is available during process → earlier decision on experimental consequences are possible

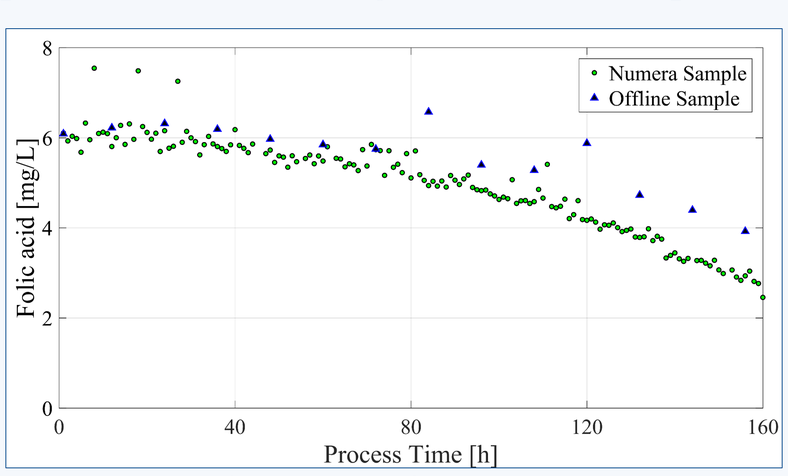
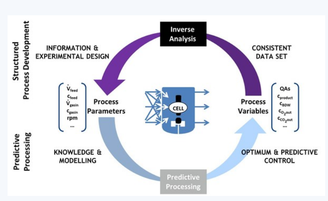


Figure 4: Time course of the concentration of a vitamin in the supernatant. Automated sampling (green dots) was performed every hour and manual sampling (blue triangles) every 12h.

CONCLUSION

High frequent sampling and precise analysis of these samples via HPLC facilitates **process transparency** and is necessary for **process understanding** e.g. for investigation of limiting components and media development. In order to reduce time and cost in process development and production timely availability of these HPLC data is indispensable. **Automated sampling and sample processing** can lead to **faster detection** of the process **end point** of production as well as to faster evaluation of **limiting or inhibiting components** for media development.
The actual sampling frequency that is necessary for these applications as well as for multiple bioreactor systems has to be still evaluated.



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