Bioprocess Sampling

A Necessary Evil or a Chance for Improved Monitoring and Control?

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Monitoring of biopharmaceutical processes

Monitoring and control of critical process parameters (CPPs) in various bioprocesses is a prerequisite to gain a safe product and hence, is a major topic in Process Analytical Technology (PAT) [1]. The most widespread methods for process monitoring are linked to sampling. Sample-based monitoring has various advantages: direct measurement of specific analytes; usage of highly automated offline analyzers; reliability through application of reference methods; extensive parallelization; flexibility and storage of reference samples for later analysis [2].

Due to these benefits, sample-based monitoring is still state of the art and commonly combined with standard PAT tools (i.e. in-line sensors for pH, pO_2, T) for bioprocess control. Advanced PAT tools such as offgas analyzers, dielectric spectroscopy, IR or Raman have evolved considerably over the last decades. Com-

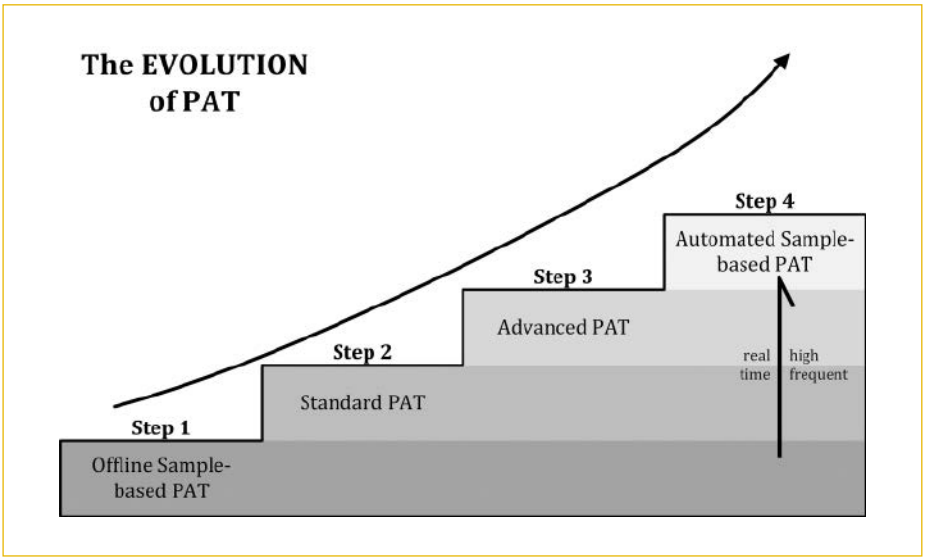

Fig. 1: PAT tools have made evolutionary steps over the last decades. Manual sample-based monitoring is still the basis due to various advantages like flexibility and direct measurement of specific analytes. The combination with standard PAT tools like pH, pO2 or T probes is state of the art. Advanced PAT tools such as offgas analysis or spectroscopic methods have evolved bioprocess control but could not replace sample-based methods. Hence, the next evolutionary step in PAT leads to automated sample-based monitoring; transforming offline methods to online methods.

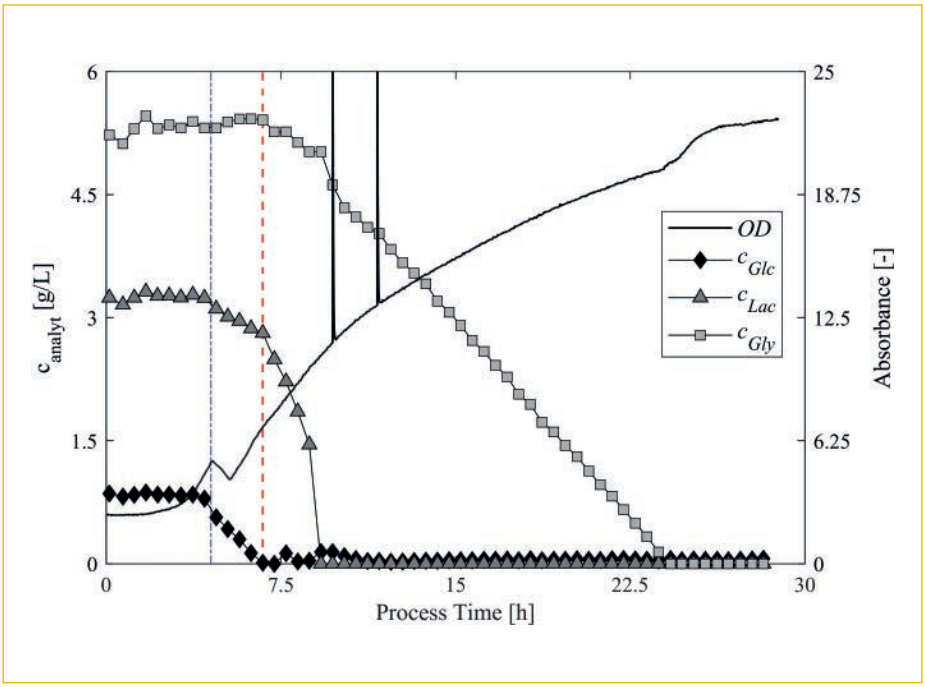

Fig. 2: Time course of an *E.coli* fermentation using auto-induction media. The process was monitored by inline OD (black line) as well as automated sampling combined with online HPLC for detection of glucose (diamonds), lactose (triangles) and glycerol (squares). The blue line (· -) indicates the time point for the temperature shift according to the inline OD and the red line (- -) according to the direct measurement of the primary substrate glucose and the inducer lactose. A delay of over 2h depending on the applied method can be observed.

bined with statistical methods for signal post-processing or model-based soft-sensors, increasing amounts of information can be extracted from the online signals and the overall understanding of bioprocesses is further improved. However, these tools do not allow for replacing sample-based monitoring, hence it is still a central task for sound in-process control (fig. 1).

Despite its essential importance, manual sampling holds some major drawbacks: i) the invasive method bears a risk for contamination of the process and sample, ii) the high sampling volume needed, especially for small parallelized bioreactor systems, iii) the operator-to-operator deviations during sampling, sample preparation as well as analysis, iv) it is tedious and requires a lot of human resources, v) low sampling frequency and commonly no real-time availability of data and vi) paper-based and error-prone data management.

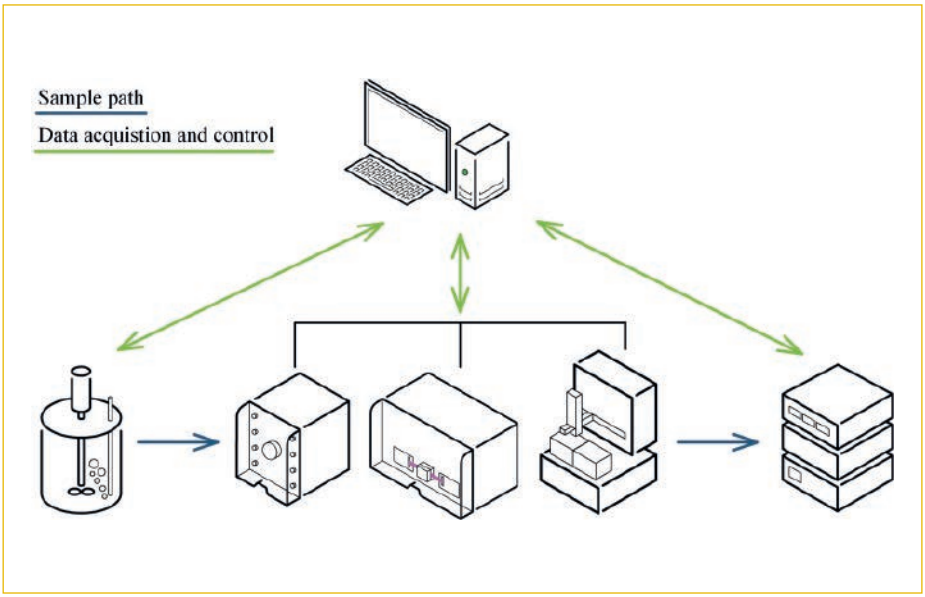

Fig. 3: Automated sample-based monitoring requires reliable and robust sampling, analytics, data management and data processing. The applied setup of the case study meets these requirements, which is shown schematically. The multiplexer module is drawing the sample from the bioreactor, which is processed in the filtration module and stored in the autosampler (Numera, Securecell AG). By an injection valve a part of the sample can directly be injected in the HPLC system (1200, Agilent). An overarching software (Lucullus PIMS, Securecell AG) is controlling the bioprocess, triggering sampling and analysis, aligning analytical and process data and finally applying feedback control.

Next Evolution in PAT

Taking all these advantages and disadvantages into account, what is the next evolutionary step for PAT tools? Sample-based monitoring will stay a non-evitable part in process monitoring. However, with the advent of digital tools in biotechnological R&D and production floors, there is considerable potential to be gained via automation of sampling, sample preparation, analysis and sample and data management. Sophisticated automation allows for reliable and sterile sampling of the minimal required volume. All steps from sampling to analysis are then reproducible without the risk of human errors. High frequency sampling and real-time data availability can be achieved without the need of additional human resources. In addition, it still holds all the advantages of typical, manual sample-based monitoring like direct measurements, storage of reference samples and multiplexing for parallel approaches, which are increasingly applied in process development.

In the following case study an example for the superiority of automated sample-based monitoring is demonstrated.

Case Study

In the illustrated case study an *E.coli* process was monitored via an advanced PAT tool, namely an inline sensor for optical density (OD) and via automated sample-based monitoring using an online HPLC. The signal was used as a basis to trigger a process event, in this case a temperature shift. The process was performed using an auto-induction medium, designed for sequential consumption of glucose and glycerol as C-source and lactose as induction reagent for protein production. The induction starts after the consumption of glucose and with the onset of lactose metabolism, which indicates the desired time point for the temperature shift. A widespread approach for determining the time for the temperature shift is the repeated (offline) measurement of OD in a cuvette. Historically, this is the most readily available growth-related variable that is easy to

measure and empirically assessed. Due to the afore mentioned disadvantages of manual sample-based monitoring, advanced PAT tools (i.e. inline OD) can be applied. They deliver a high frequent signal however, with low information content. In figure 2, it can be clearly seen, that the time point for the temperature shift according to the absorbance at 4.5 h is not associated with the complete consumption of glucose. In addition, this signal is highly depending on cell physiology, hence, the correlation to the biomass changes with e.g. changing cell size, leading to a variable signal over different processes which is not optimal for control actions. Alternatively, automated sampling automated sampling allows a direct online analysis of the sugars by HPLC. In comparison to manual sampling a high frequent and reliable analysis is possible delivering a concentration value every 30 min. The automated monitoring approach leads to a drastic delay of 2h of the optimal time point for a temperature shift compared to the inline OD probe. Taking the sampling interval of 30 min into account, this resembles a reduction of an error of the correct time point of 75%. The automated sample-based approach allows for direct analysis of the targets describing the cell metabolism in comparison to the measurement of a substitute value like OD.

Automation and Digitization

In order to realize a process event based on a sound knowledge based control strategy, two requirements have to be fulfilled: i) reliable data based on reliable PAT tools and ii) a software to enable data management including capturing of process as well as analytical data, alignment of the data from different sources, evaluation of the data and finally application e.g. in form of a control action like the initiation of the temperature shift. In the above example these two requirements were fulfilled by the combination of an automated sampling system (Numera) enabling online HPLC analysis (Agilent 1200) and a Process Information Management System (Lucullus PIMS) (fig 3).

The automated sampling system is a modular system consisting of a multiplexer module for sterile sample drawing, a dilution and a filtration module for sample preparation and an autosampler for sample storage and transfer. The transfer is realized by an injection valve at the sampler that is directly connected to the pump and the column oven of the HPLC. The sample trigger as well as the trigger for HPLC analysis is initiated by time in the Process Information Management System. This software can directly start the HPLC measurement by starting an injection in the HPLC software (ChemStation, Agilent). After the run, the HPLC software is processing the peaks as usual and the concentration results of the analytes are directly sent back to the Process Information Management System. There the results can directly be used for triggering a process event. In the above described case, an average value of the first five results of the glucose and the lactose measurements was calculated in the Process Information Management System. If a newly generated value of both substrates was below 10% of the mean values a temperature shift was performed automatically. The temperature was decreased from 37°C to 25°C within 45 minutes.

Future Perspectives

Automated sample-based monitoring and control as a reliable PAT tool is a platform technology and paving the way for the bioprocesses of the future. The possibilities of at-line analytics are brought closer to the process. This will lead to unprecedented possibilities in bioprocess engineering by determination of the target of interest itself instead of substitute values.

Simultaneous sampling of different bioreactors will support further parallelization and finally shorter development times. In addition, the real-time availability of all necessary process information will revolutionize the bioprocesses itself. Based on the direct monitoring of novel CPPs or directly critical quality attributes (CQAs), control strategies from other scientific fields could be applied in biotechnology. This will support the trend to continuous manufacturing.

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