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Application note
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Glucose monitoring and control using Numera®, Lucullus® PIMS and Cedex® Bio HT Analyzer

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Abstract

Critical process parameters (CPPs) such as glucose must be monitored and controlled in every bioprocess in order to reach the desired product quality in the most efficient way. Still, there is a lack of tools to bring the analyzers and their results closer to the process. This application note shows how the combination of two technologies, namely the automated sampling and sample processing system Numera® and the Process Information Management System Lucullus® (Lucullus PIMS), enable on-line and real-time availability of data for monitoring and control purposes. Both glucose monitoring and control are demonstrated in a CHO process and a technical experiment.

Introduction

The goals of every bioprocess are i) to produce a valuable product with ii) a robust and efficient process that iii) delivers a predefined product quality. To achieve this goal regulatory guidelines have shaped the concept of critical process p rameters (CPPs), key process parameters (kPPs) or critical material attributes (CMAs), which have an impact on process performance and product quality [1]. These parameters and attributes must be identified and characterized by adequate an lytical methods for every process. The CPPs must then be monitored, so as to obtain the analytical results on-line and in real-time, which enables sc ence-based process optimization and control (Figure 1). New technologies can hereby support deeper process understanding and help to reach the afore mentioned goals of bioprocessing [2].

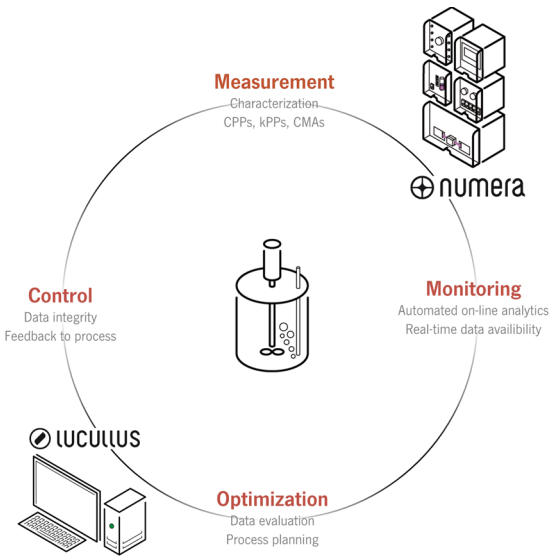

Figure 1: Science-based process design can be achieved by following the presented loop. Within the loop, the process must be characterized for critical process parameters, key process parameters and critical material attributes; and be monitored, optimized and controlled.

Common reference methods for the analysis of critical attributes are HPLC methods or enzy matic analyzers such as the Cedex® Bio HT, which are hardly available in an on-line mode. In order to use these devices on-line, the gap between the pr cess and the analyzer must be closed by a liquid ha dling device as well as by adequate data transfer and data integration in the process management system. Thus, the loop comprising measurement over monitoring and optimization to control as shown in Figure 1 would be enabled. The combination of Numera® and Lucullus® PIMS precisely enables this loop and fills the afore mentioned gaps. Numera® is an automated sampling and sample processing system that can be connected to various analyzers. Lucullus® PIMS is a Process Information Management System (PIMS) merging all process relevant data in one system, including tools for process optimization and control based on the analytical data. Examples of the possibilities that the combination offers are given in a CHO process and a cellfree technical run using the Cedex® Bio HT Analyzer.

The applied system

Numera® and Cedex® Bio HT

The automated sampling system Numera® is equipped with the following modules: a multiplexer module, a dilution module, a filtration module, a routing module and an autosampler. The autosampler is used to collect the samples and to store them at 4°C. The routing module fulfills two major tasks: i) it enables individual sample processing (i.e. individual combination of dilution, filtration or neither of both) for each sample in one process and ii) it facilitates the sample transfer from the autosampler to the Cedex® Bio HT or to other analyzers. Initiation of a sample measurement by the Bio HT is achieved with a software interface between Lucullus PIMS and the Bio HT control software (System Version 5.0.0.1206). The sample is drawn, processed and deposited in the autosampler by Numera. Subsequently the transfer to a fixed position in the racks of the Bio HT via the routing module is performed.

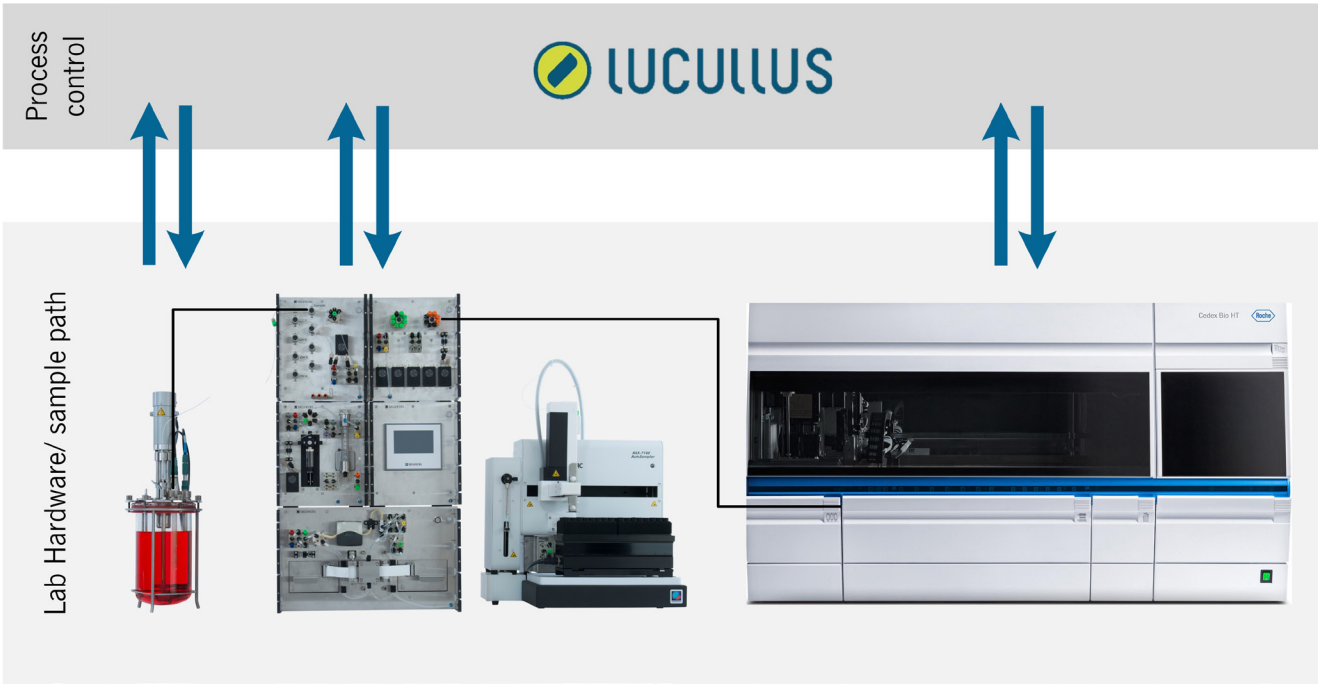

Figure 2: The laboratory infrastructure consists of two major levels: i) the lab hardware and ii) the software to control the systems. The applied hardware consisted of a bioreactor system (including pumps, balances etc), Numera equipped with a multiplexer, dilution, filtration and routing module as well as an autosampler and Cedex® Bio HT. The sample path for automated sampling and analysis is marked by the black line. On the software level only Lucullus is required, which facilitates the communication between all available devices as well as control actions. No additional middleware is required.

Once the sample is placed there, Lucullus® – providing an integrated Lucullus® PIMS initiates the measurement via the Bio HT control software.

Lucullus® PIMS

Lucullus PIMS has all basic functions of a process information management system and more: it can be used for the design, preparation, execution and evaluation of various processes. Additionally, it takes over the communication between Numera and the Cedex® Bio HT. Hence, it provides the sample management interface and initiates the samples accordingly on both platforms (numera and BioHT). The measurement results are transferred back to Lucullus® PIMS and can be displayed and processed along with other data such as pH, temperature or offgas. Hence, no additional middleware is required for transporting the analytical results to the process (Figure 2).

Case Study: Goal

Demonstrating the application of Numera® and Lucullus® PIMS for providing on-line data from Cedex® Bio HT for monitoring and control purposes.

Materials and Methods

Set-up

The set-up consists of a 3.6 l bioreactor that is connected to the Numera® system and the Cedex® Bio HT Analyzer from Roche Costum Biotech. The reactor is equipped with a pH and a pO2 probe and supplied with air, O2 , CO2 and N2 to control disolved O2 and CO2. All devices are connected to Lucullus® PIMS.

CHO process

The monitoring application is demonstrated with a CHO batch process using complex media. pO2 and pCO2 were controlled at 40% and 12.5% respectively. pH and temperature were controlled at 7.00 and 37°C. Automated sampling and analysis by the Cedex® Bio HT were performed every 2h or every 30 min.

Technical run

A cell-free technical run was performed to demonstrate the feasibility of controlling glucose based on the on-line Bio HT data. The reactor was filled with a glucose solution and constantly fed with a lactate solution in order to simulate a CHO batch process. After 9.5h, a glucose feed was started. After a short phase without measurement, the online analysis was initiated again. A simple PID controller was implemented in Lucullus® PIMS to control glucose on a level of 10mM. The control is based on the interaction between all devices. Numera takes a sample, which is analyzed by the Cedex® Bio HT. The result is transferred to Lucullus PIMS, which adapts the pump rate of the glucose feed accordingly.

Results

During the CHO batch phase glucose, lactate, ammonium and product (IgG) were analyzed by the Bio HT. If the analyte was over the test range, an automatic dilution was performed by the Bio HT, which is important for glucose in the beginning of the batch phase and for lactate later in the batch phase. If the analyte was under the test range, no result was transferred to Lucullus® PIMS. This is important if the data is used for control purposes. In the batch process, sampling and analysis were performed every 2h, monitoring the trend of the afore mentioned analytes. In addition, manual samples were drawn every 8h as a reference. Both measurements were in good accordance, demonstrating the reliability of the automated system (Figure 3A). During one batch, the sampling frequency was increased to 30 min in order to demonstrate the possibilities for monitoring (Figure 3B). With the applied software version of the Bio HT, 30 min represents the lowest possible sample interval as no feedback of the Bio HT status is available in Lucullus PIMS. In summary, the process of drawing a sample – including sample filtration – takes 8 min, transferring the sample approximately 4 min and measurement of the Bio HT up to 19 min. As the set-up could be nicely applied for monitoring, a

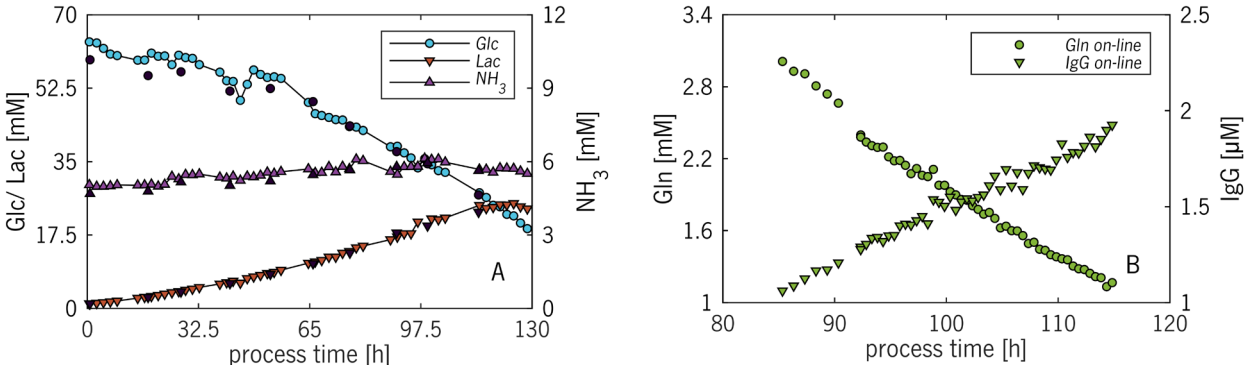

Figure 3: Plot A illustrates the results of monitoring glucose (light blue), lactate (red) and ammonium (violet) during a CHO batch process. The corresponding at-line data of the manually drawn samples are displayed in black. Plot B shows a phase of highfrequency on-line measurements of glutamine (green circles) and product (green upside-down triangles) in the CHO process.

cell-free control run was performed as described above. The experiment demonstrates that the set-point of the feed pump stays unchanged if no result is received by the analytical device. If a result is received, a simple PID controller is sufficient to i) regulate the concentration to the desired setpoint and ii) control the concentration on the desired setpoint with a deviation of about 3% (Figure 4).

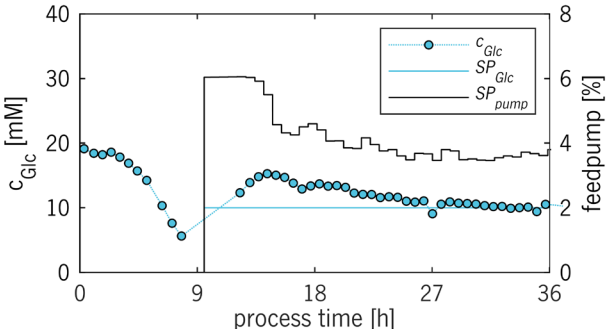

Figure 4: The process was divided into three phases: the phase simulating the batch till 9.5h, the feeding phase without control till 12h and the controlled feeding phase starting from 12h.

Conclusion

The connection between the Cedex® Bio HT Analyzer and the bioreactor system was successfully established by applying Numera® for sampling and sample processing and Lucullus® PIMS as overall software for data management and control. The installation as an enabler for substrate, metabolite and product monitoring was demonstrated with a CHO process. The minimal sampling and analysis interval in the demonstrated applications was 30 min. With the available software update of the Cedex Bio HT Analyzer Application Software (Version 5.1.1.1801), the interval could be further reduced as the new version supports feedback communication with Lucullus® PIMS. Furthermore, a technical run showed that control on a specific target concentration is possible with a deviation of about 3%. The low control error was reached using a simple PID controller. Of course, more sophisticated controllers, including model-based methods, could be applied. In summary, it is shown that the combination of Numera® and Lucullus® PIMS closes the gap between the process and the analytical stream. Hence the loop from measurement over monitoring and optimization to control (Figure 1) is put into practice.

Key Results

– Successful monitoring of substrates, metabolites and product
– High sampling frequencies of 30 min (including sampling, analysis and availability
– of analytical results in PIMS)
– Control of glucose concentration enabled by the combination of, and interaction
– between Numera®, Lucullus® PIMS and Cedex® Bio HT Analyzer

References

[1] Guideline IHT (2011). "Development and manufacture of drug substances (chemical entities and biotechnological/biological entities) Q11", London: European medicines agency
[2] Kroll, P. et al (2017), "Model-Based Methods in the Biopharmaceutical Process Lifecycle", Pharmaceutical Research, 34 (12), 2596–2613

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