Numera® & Lucullus®: an integrated PAT solution combining automated sampling and process control

Alexandra Hofer Securecell AG, In der Luberzen 29, 8902 Urdorf, Switzerland (alexandra.hofer@securecell.ch)
Paul Kroll Securecell AG, In der Luberzen 29, 8902 Urdorf, Switzerland (Switzerland (paul.kroll@securecell.ch)
Christoph Herwig TU Wien, Gumpendorferstrasse 6a, 1060 Vienna, Austria (christoph.herwig@tuwien.ac.at)

Abstract

This application note presents two unique PAT tools, which can be used separately or together as an integrated PAT solution. The Numera® system enables automated sampling, sample preparation, sample storage and even on-line analytics. The sampling is triggered by Lucullus® PIMS, which also collects the analytical data and hence unites all data attached to a process in one software. Lucullus® PIMS supports planning, preparation, execution and evaluation of any kind of process. Additionally, it enables feedback and process control. The combination of both tools enables to design, analyze and control a process according to PAT requirements as demonstrated with a tech-run experiment. Hence typical tasks in bioprocess development are facilitated and accelerated.

Introduction

Process Analytical Technology (PAT) is still an emerging topic but a partly unfulfilled task in the bioprocessing environment. Sampling, sample processing and sample analytics are important topics within the PAT framework. Critical process parameters and product quality attributes must be measured, monitored and controlled [1]. For most reference analytics, sampling and sample processing are required, which holds a risk for contamination and can be error prone, e.g. operator-to-operator errors or analytical variability. Automation of sampling can overcome those disadvantages and additionally deliver samples in high frequency and from different bioreactors directly to the analytical stream. On-line connections to different analyzers, such as HPLC, are paving the way from simple measurement to automated monitoring.

In addition, PAT not only aims for analytics but also for an integrated solution that provides a framework for collecting, evaluating and managing all data attached to a process. Furthermore, the loop between gathered data and the bioreactor must be closed to allow feedback for process control. All these requirements can be combined in a process management system that merges all data – from on-line data such as pH or temperature to at-line or off-line information such as sampling time and corresponding analytical results – and hence facilitates data integrity and the possibility of feedback for advanced process control. Both tasks – automated sampling as well as data management – can be fulfilled by our integrated PAT solution combining Numera and Lucullus® PIMS.

The PAT tools

Numera® – automated on-line sampling and more

Numera® is a modular system that allows automated sampling, sample preparation, sample storage and on-line analytics of up to 16 bioreactors in parallel. One multiplexer module can sample 4 bioreactors in parallel and includes the sterility barrier of the system. The immersion nozzle for sampling is attached to the bioreactor during autoclavation and can afterwards be easily connected to the multiplexer module. Hence, no vapor sterilization or similar is required after sampling leading to reduced requirements for the lab environement and increased safety. The sampling volume is as low as possible, which is approximately between 2.5 and 4 mL to generate maximum information per mililiter and it can be adapted to the required analytics. Excess of sample is pushed back into the reactor via sterile air in order to save sample volume as well as to avoid cross-contamination of samples. The dilution module allows dilutions between 1:1 and 1:30 with water or the initiation and performance of certain reactions by dilution and incubation with a specific reagent. The filtration module is equipped with a continuous filter band that uses a new piece of filter for every sample taken by the system. This novel approach prevents filter clogging or cross-contaminations. All drawn samples are transferred to an autosampler, which can be equipped with 1.5 to 10 mL vials. The samples are stored there at 4 °C or they can be further transferred to an analytical device (Figure 1).

As this is a modular system, all modules can be used independently. Therefore, a sample can just be drawn, additionally diluted or filtered as required by the subsequent sample analysis.
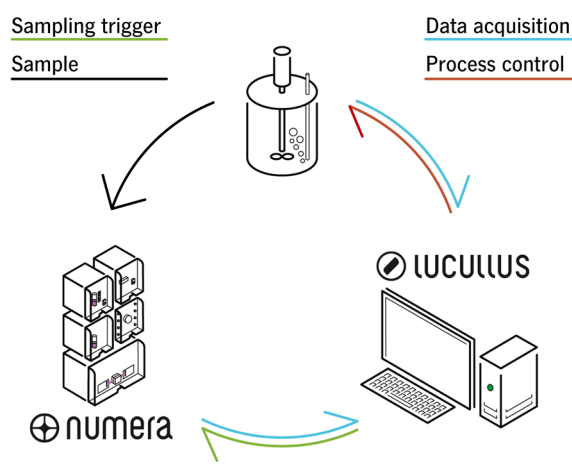

Figure 2: Interaction between Lucullus® PIMS and Numera®. Lucullus® PIMS is receiving data from the bioreactor and all connected devices such as the automated sampler (blue arrows). A sampling trigger can be set in Lucullus® PIMS and the command is forwarded to the Numera® system (green arrow). A sample is automatically taken and processed by Numera® (black arrow). Process control, e.g. feeding, can also be achieved via Lucullus® PIMS (red arrow).
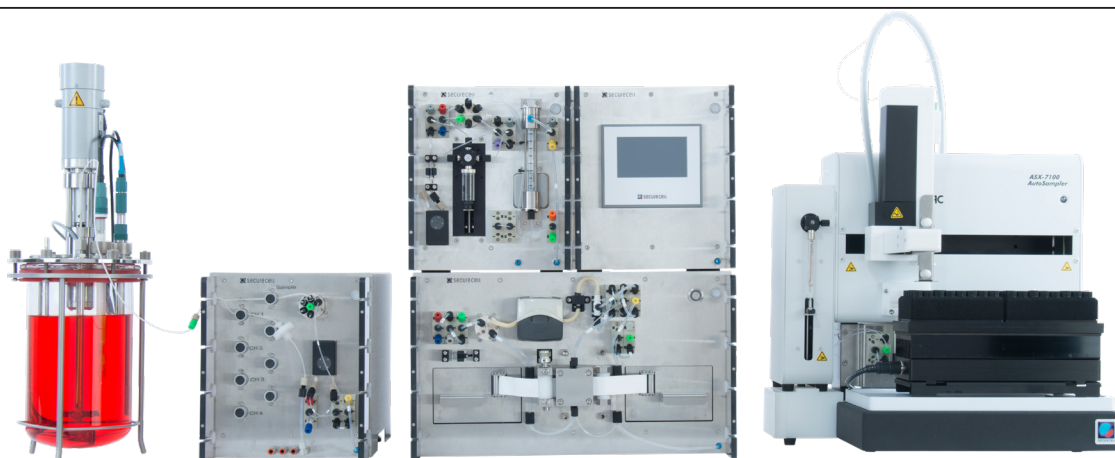

Figure 1: Numera® for automated sampling including a multiplexer, dilution and filtration module as well as an autosampler for sample collection and storage along with a control module. The whole device is integrated in the process management system Lucullus® PIMS.

Lucullus® PIMS – providing an integrated PAT solution

In order to apply an automated sampling system in a meaningful manner, the software used for the sampling trigger is crucial. Commonly, there is an independent software for this task, which may be connected to the Process Information Management System (PIMS) by OPC. Without this connection, the sampling trigger cannot be associated with process events, and vice versa, and the analytical results cannot be fed back to the process. To solve these problems without extensive software patchwork, Lucullus® PIMS provides a single solution. Lucullus® PIMS can either be applied to simply trigger the sample or be used as PIMS for the whole process. This way, the sampling trigger can be set by time or be attached to a process phase (Figure 2). Additionally, the sampling trigger can be created during the planning phase of the experiment, allowing to reduce human errors on creation of sample triggers right in the planning phase. As a process management system, Lucullus® PIMS supports design, preparation, execution and evaluation of whole bioprocesses and enables integrated bioprocesses as single software solution.

Case Study: Goal

The automated sampling system is evaluated for accuracy and precision, sterility and off-line usage. In addition, the basic functionality of the process management system is demonstrated.

Materials and Methods

Set-up automated sampling

The Numera® system is equipped with a multiplexer, a dilution and a filtration module as well as an autosampler. In the presented application, the sampling system is applied for sample storage only. The samples are afterwards analyzed by HPLC or the Cedex® Bio HT (off-line).

Accuracy and precision

Both parameters were experimentally assessed: different glucose standards were prepared, directly analyzed by a Cedex® Bio HT (Roche Diagnostics) or first processed by the Numera system in triplicate and subsequently analyzed. The samples were processed through all available modules, without applying a dilution.

Sterility and off-line usage

Sterility and off-line usage are demonstrated in a technical run , i.e. without cells, with dynamic conditions. The set-up consists of a 3.6 l bioreactor equipped with a sampling nozzle that is connected to the Numera® sampling system. A water and a glucose feed are connected to the bioreactor, which are controlled via scales and pumps. All devices are connected to Lucullus® PIMS.

The technical run was performed under sterile conditions, mimicking ideal conditions for microbial growth. The reactor was filled with 1.4 L medium [2], pH was set to 7.00, temperature was
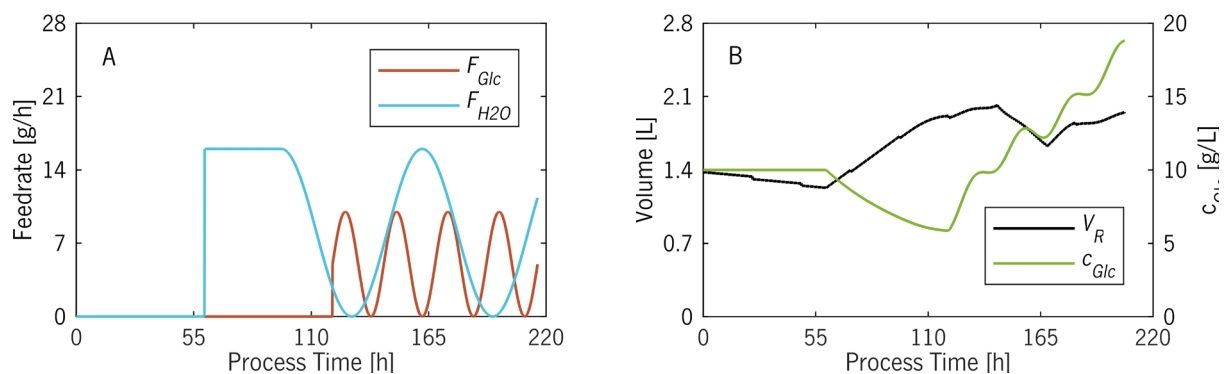

Figure 3: The process simulation of the water and the glucose feed is shown in plot A. Plot B illustrates the reactor volume and the expected glucose concentration in the vessel.

set to 37°C, pO_2 was set to 40% and pCO_2 was set to 12.5%. The water feed was started after 60h as constant feed for 36h, followed by sinus feeding till the end of the process. The glucose feed was started as sinus feed after 120h. Automated samples were drawn every 2h and manual samples were taken every 24h. A simulation in Matlab® 2017a was performed in order to estimate volume change and the glucose profile over process time (Figure 3). An operation to control the water and the glucose feed was implemented in Lucullus® PIMS. The dynamic setpoints for the feed rates were calculated by the following equation:

$$r_j = \frac{a_j}{2} \cdot \left[\sin(f_j \pi t) + 1 \right]$$

with

$$a_{Glc} = 10 \left[\frac{mL}{h} \right] ; \; f_{Glc} = \frac{1}{12} [h^{-1}] \bigg|$$

$$a_{H2O} = 15 \left[\frac{mL}{h} \right]; \; f_{H2O} = \frac{1}{33} [h^{-1}]$$

The planning tool of Lucullus® PIMS was applied to book the needed bioreactor, to choose the right operation and to order the manual and automated samples along with the required analytics.

Results

Accuracy and precision

A prerequisite for the application of automated sampling is that the analytical results remain accurate and precise. Figure 4 shows the linear correlation between glucose standards and processed glucose samples by the Numera system. A slight offset can be noted in the slope of 0.95, which is caused mainly by the dilution module. However, the precision is very high with an error of 0.4%. This high reproducibility allows calibration of the dilution module, meaning that the accuracy can be increased if dilutions are performed by the system. Summarizing, adequate accuracy and high precision of the system are approved – no unpredictable bias of the analytical data is created by the system.
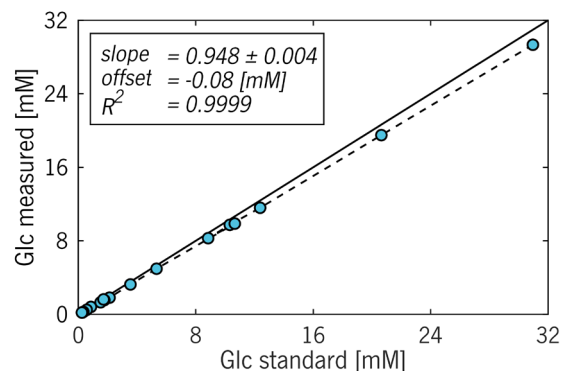
Figure 4: Directly measured glucose standards (x-axis) and the same samples processed with the Numera system (y-axis) are in good accordance with an R^2 of 0.999.

Sterility and off-line usage

The technical run in the bioreactor could be performed as planned, meaning that the aimed feed control was executed by Lucullus® PIMS as planned in the process simulation (Figure 5A).
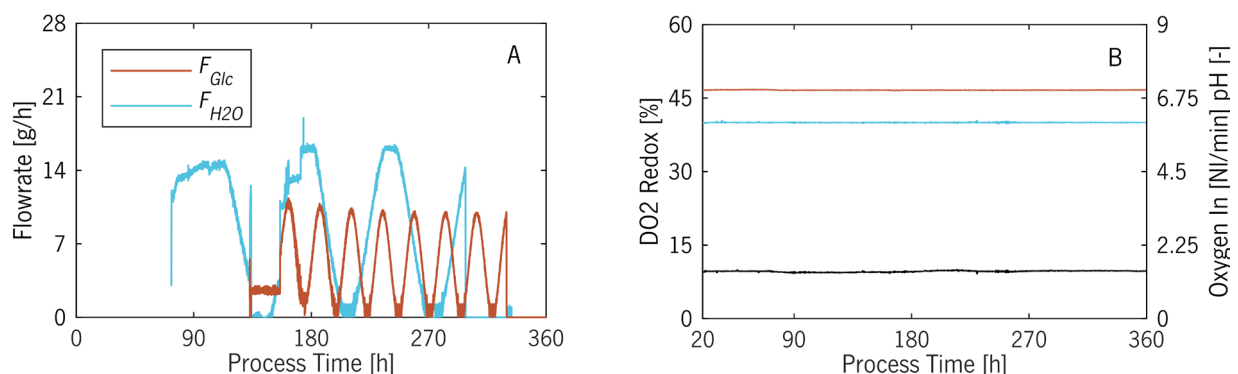
Figure 5: All data attached to the process is available in Lucullus® PIMS. Plot A shows the actual feed rate of the water and the glucose feed. Plot B shows the values for pH (red line), pO_2 (blue line) and O_2 inflow (black line) over process time. The steady values indicate a sterile process over 370 h.

Additionally, all data concerning pH, pO_2, gas inflows etc. are easily accessible and imply that the system was sterile over 360h (Figure 5B).

Automated samples were drawn every 2h and manual samples were taken every 24h. All samples were stored until the process was finished and then analyzed by HPLC. As the initial medium contained different sugars that are not completely separated in the chromatographic method, a certain minimal offset between the concentration predicted by the model and the measured values is visible. It should be noted that the change in glucose concentration can still be seen nicely with the automated sampling, whereas the manual samples indicate a different glucose pattern over the process (Figure 6).
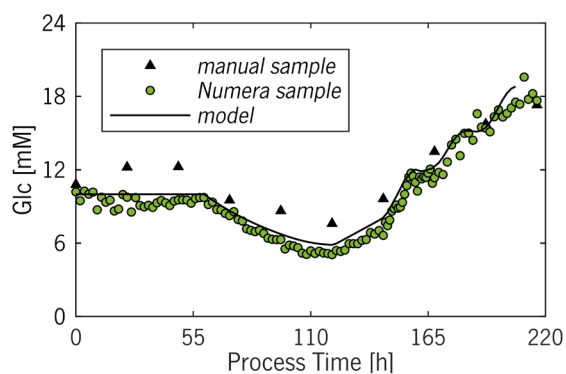

Figure 6: Comparison of the glucose concentration in the bioreactor according to the model (black line), the Numera samples (green dots) and the manual samples (black triangles).

Conclusion

Numera® and Lucullus® PIMS are a powerful combination that meets the criteria for an integrated PAT solution. Accuracy and precision as well as long-term sterility of the automated sampling system have been proven. Furthermore, the modular design of Numera® allows individual applications and easy handling (no clogging of membrane filter, no sterilization necessary after sampling). Lucullus® PIMS allows design, preparation, execution and evaluation of whole processes, demonstrated by the example of sampling trigger for the automated sampling system as well as by the example of a sinus feed control.

In combination with Lucullus® PIMS, Numera® can be connected to numerous third-party analytical devices in order to facilitate advanced process monitoring and control.

Key Results

– Long-term sterility
– High precision
– On-line and off-line reference analytic
– Powerful PAT-tool
– Process transparency

References

[1] FDA (2004). "Guidance for Industry PAT-a Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance." www.fda.gov
[2] Kroll, P. et al (2017), "Workflow to set up substantial target-oriented mechanistic process models in bio-process engineering", Process Biochemistry, 62, 24–36

CONTACT	For more information on our products and services visit our website at	Securecell AG In der Luberzen 29 8902 Urdorf Switzerland
	www.securecell.com	+41 44 732 90 70 contact@securecell.ch