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Application note
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Lucullus® PIMS for event-based process control

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Abstract

Process control is a central task in bioprocess development and production. Apart from common control tasks including pH, temperature or dissolved oxygen, more advanced control tasks must be mastered. Real-time availability of analytical data is often the basis to achieve a control based on process understanding. This application note demonstrates how sophisticated control tasks can be easily implemented in the Process Information Management Software Lucullus® PIMS. Exemplary, a temperature shift in an Escherichia coli (E. coli) process is automatically performed based on different on-line signals available in Lucullus® PIMS: i) optical density and ii) glucose and lactose concentration determined by automated sampling and sample preparation via Numera® followed by on-line HPLC analysis. Both approaches are compared with respect to the current state of the culture as well as their impact on process robustness.

Introduction

The biological origin of bioprocesses leads to high complexity, high variability and sometimes inefficient production. Hence, automated and science-based control strategies are essential to design robust processes. The basis for such control strategies is an overarching software that i) integrates all devices and sensors attached to a process, ii) structures and records all data along the processes and iii) allows performing individual control actions based on the incoming data in a reliable and reproducible manner. This application note gives two examples of automated event-based process control in an exemplary Escherichiacoli (E.coli) process using the pET expression system and an auto-induction medium. This medium exploits the sequential uptake of different carbohydrates by combining primary and secondary substrates for cell growth and energy supply with lactose for protein induction. Typically, a temperature shift which should coincide with the start of induction is performed in such auto-induction processes. In the first example this shift is performed based on an in-line optical density (OD) signal. In the second example, the consumption of glucose and lactose is monitored via automated sampling and sample preparation with Numera® combined with an on-line HPLC analysis. The trigger for the shift is directly based on the substrate consumption behavior of the culture. Finally, both methods are compared regarding the overall goal of reaching a robust process design with the applied control strategy. In both cases, the Process Information Management System Lucullus® (Lucullus® PIMS) is applied for process control and automated initiation of the temperature shift.

The PAT tools

Lucullus® PIMS

Lucullus® PIMS is the software for efficient enterprise-wide bioprocess control and data management. All pieces of equipment can be integrated in Lucullus® PIMS to allow easy interaction for monitoring and control purposes. It enables design, preparation, execution and evaluation of whole bioprocesses. The devices providing the data basis for the event trigger in the E. coli fermentation were integrated in Lucullus® PIMS, i.e. the in-line OD probe and the Numera® system for automated sampling including the connected HPLC system. The Numera® system is designed as an integrated PAT solution in combination with Lucullus® PIMS, meaning that Lucullus® triggers the sampling process as mwell as the automated analysis by HPLC. The analytical data is directly transferred back to Lucullus® PIMS and available for monitoring or control. Based on the monitored data, Lucullus® PIMS performs the necessary control actions in a fully automated way. In summary, Lucullus® PIMS provides a single software solution, managing all data for integrated bioprocesses (Figure 1).

Numera® connected to Agilent HPLC system

Numera® is a modular PAT system that allows automated sampling, sample preparation and transfer to an analyzer. For HPLC connectivity, Numera® is equipped with a Multiplexer Module, a Dilution Module, a Filtration Module and an Autosampler, which stores the drawn samples. In addition, the injection valve of the Numera® autosampler enables direct injection into the HPLC system. The corresponding HPLC system Agilent 1200 is equipped with a pump (1200 series), a column oven (1200 series) and a refractive index detector (1260 infinity series).

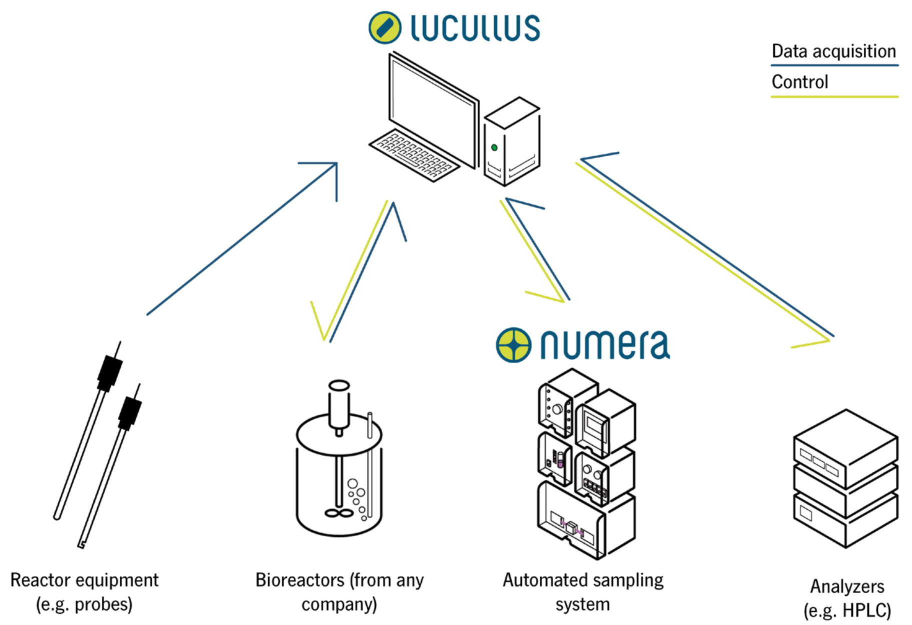

Figure 1: Lucullus® PIMS is the central software to enable data acquisition from any device, starting from bioreactors over in-line probes, Numera® for automated sampling to analyzers. In addition, advanced control tasks (including calculations) can be performed by using the real-time available data in the process step chains/ control recipes. Lucullus® PIMS is also applied to trigger automated samples as well as automated analysis of samples.

Materials and Methods

Cultivations

The fermentation runs were performed in a stainless-steel reactor which was equipped with temperature, pH, pO2 and OD880 probes, scales, pumps and an offgas analyzer. In addition, Numera® and the on-line HPLC system were connected. All devices from the reactor system over probes to Numera® and the HPLC system are connected to Lucullus® PIMS. All cultivations were performed with auto-induction media, consisting of a mixture of glucose, glycerol and lactose. An automated temperature shift was performed from 37°C to 25°C with a ramp over 45 min. The temperature shift was triggered by the OD880 from the in-line probe or by the sugar data from the on-line HPLC.

Automated trigger via step chains

The processes were designed in Lucullus® PIMS. In the Operation Tool, step chains can be easily implemented and modified to adapt process control (Figure 2).

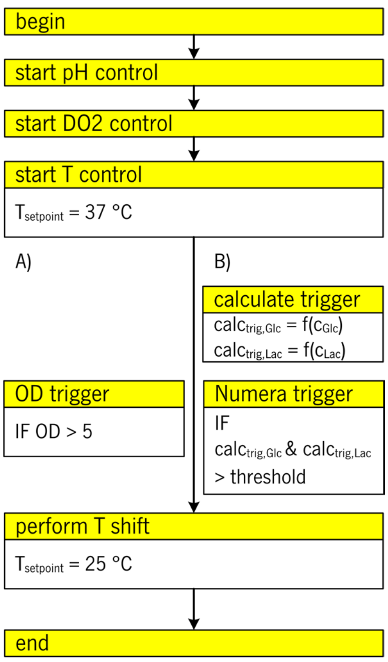

Figure 2: Implemented step chain for A) OD-triggered temperature shift and B) Numera® triggered temperature shift.

Automated trigger using the in-line OD880

Lucullus® PIMS receives a signal from the in-line OD about every 5s. The procedure for the automated temperature shift can be seen in the step chain (Figure 2A): If the OD880 signal rises above the value of 5, the temperature shift is automatically initiated.

Automated trigger using the Numera® system

The HPLC method for sugar analysis resulted in a total sampling and analysis time of 30 min. The 30 min included the initiation of the sample, filtration, transfer to the HPLC, HPLC analysis and data transfer back to Lucullus® PIMS. Hence, Lucullus® PIMS receives an analytical result every 30 min, with a 30 min delay. The trigger point in the step chain for the temperature shift is defined as follows (Figure 2B): For the first five results of both glucose and lactose the average is calculated. The newly measured value is compared with the average values in Lucullus® PIMS. If it is 10% below the average for both glucose and lactose, the temperature shift is initiated.

Results

Two fermentations were performed executing the shift triggered by the in-line OD (F1) or the glucose and lactose values from Numera (F2). In both cases, the step chains implemented in Lucullus® PIMS enabled the automated execution of the temperature shift as planned. In F1, the OD of 5 was reached after 4.5 h initiating the temperature shift (Figure 3). In F2, sample 12 after 5.75 h reached the desired level of a decrease of 10% in both glucose and lactose. Hence, the temperature shift was triggered after 6.25 h when the analytical result was available in Lucullus® PIMS (Figure 4). It should be noted that this point in time perfectly fits to the complete consumption of glucose. Furthermore, higher starting amounts of glucose could facilitate a precise determination of the time point for the shift. It could be shown that Lucullus® PIMS is able to perform a process event as planned, irrespective of the triggering data source. However, both methods can be further compared concerning the reasonability behind the trigger or the shift. In general, the temperature shift is initiated as the induction of the culture takes place. Taking a closer look at F1, the shifting time point takes place even before the culture starts to consume the available sugars (Figure 5). This may negatively affect process duration, productivity and process robustness. Otherwise, in F2 the indirect measurement of OD is exchanged for a direct measurement of the sugar concentration by HPLC. Hence, the trigger is based on the current state of the process, resulting in a reproducible process with constant productivity and less variation in process duration.

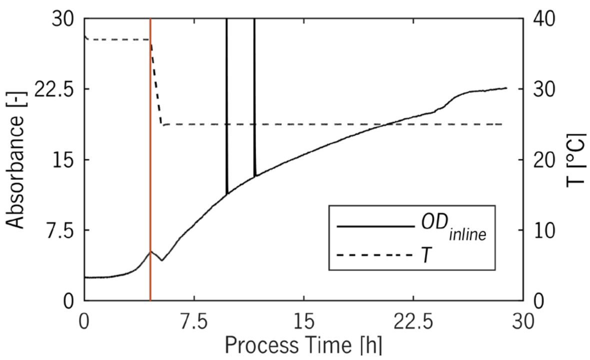

Figure 3: The in-line OD and the temperature of the cultivation can be seen over time. The temperature shift was executed as planned after an increase of the OD over 5, which occurred after 4.5 h (red line).

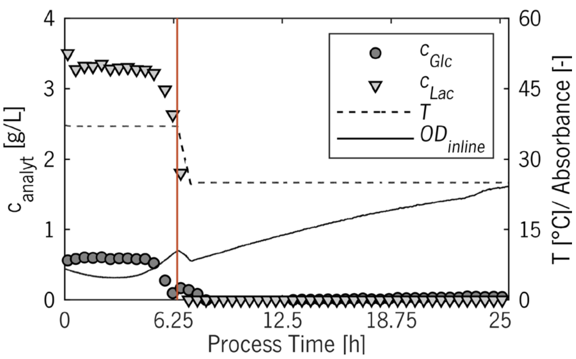

Figure 3: The plot shows the in-line OD, the temperature, glucose and lactose over time. The combination of Numera® and Lucullus® PIMS made the sugar values available in quasi real-time (30 min delay), hence a decrease of both values of more than 10% was set to trigger the temperature shift, resulting in a shift after 6.25 h (red line).

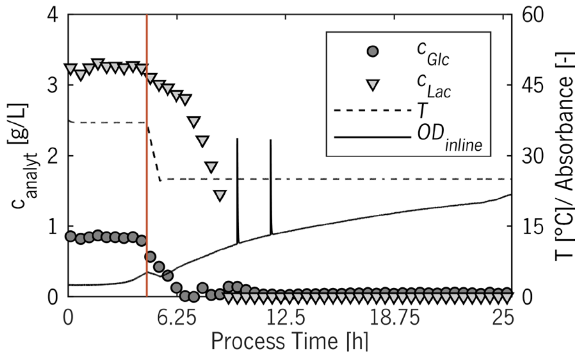

Figure 3: The plot shows the in-line OD, the temperature, glucose and lactose of F1 over time. It can be clearly seen that the time point of shift (red line) received from the in-line OD does not correlate with the complete uptake of glucose.

Conclusion

Data integration and data alignment is the basis for event-based process control. Lucullus® PIMS allows triggering setpoint changes in a user-defined manner in the step chain. The trigger can be set at threshold value of an on-line signal or be a calculation result of an on-line signal. Control strategies based on direct measurements of the analyte of interest hold a huge potential for optimizing processes in a science-based manner. Process variability can be decreased and productivity stabilized. A simple implementation is made possible by the integrated PAT solution, combining Numera® for automated sampling and on-line analytics and Lucullus® PIMS for data management and process control. Process knowledge can be generated or applied, resulting in a robust production process.

Key Results

- Data integration in one software (Lucullus® PIMS)
- Automated sampling and on-line HPLC analysis of sugars by Numera®
- Advanced process control enabled by Lucullus® PIMS
- Combination of Numera® and Lucullus® PIMS as integrated PAT solution
- Robust process design

CONTACT

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www.securecell.ch

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