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Automated Bioreactor Sampling – Process Trigger Sampling for Enhancing Microbial Strain Characterization

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Abstract

This application note describes the integration of a Flownamics Seg-Flow® 4800 Automated On-line Sampling System with Eppendorf DASGIP® Parallel Bioreactor Systems as implemented at the Energy Biosciences Institute in Berkeley, California. The automated process trigger sampling technology enabled the researchers to rapidly characterize process events, parameters and stress responses that impact yeast strain gene regulation and, ultimately, biofuel productivity.

Introduction

Scientists at the Energy Biosciences Institute (EBI) conduct research in a variety of areas in bioenergy development. The Quantitative Engineering of Industrial Yeast program at the EBI focuses on a thorough, systems-level understanding of bacterial and yeast metabolism, gene regulation, and stress response for elucidating principles to help rationally engineer bacteria and yeasts for improved biofuel production from lignocellulosic sources [1].

In order to accomplish their goals and objectives, researchers in the Quantitative Engineering of Industrial Yeast program have implemented automated processes, including the use of an integrated parallel bioreactor system and automated bioreactor sampling system, to conduct experiments for optimizing yeast strain characterization and selection.

Materials and Methods

Incorporating tools such as parallel bioreactor systems and automated bioreactor sampling technologies can significantly reduce project timelines and increase the efficiency of the microbial strain characterization and selection process.

DASGIP® Parallel Bioreactor Systems

Eppendorf DASGIP Parallel Bioreactor Systems allow for advanced screening of bacteria, yeasts and/or fungi. The multi-bioreactor/vessel design enables parallel experimentation intended to accelerate process development and increase throughput. Multiple bioreactor vessels are controlled via shared equipment and a single computer system, enabling the experimenter to test multiple conditions side-by-side or by allowing multiple independent

Fig. 1: DASGIP Parallel Bioreactor System for biofuel development

experiments to be run simultaneously using the shared equipment resources. Additionally, the DASGIP Parallel Bioreactor System's modular design provides ease of setup and maintenance, while offering the same control strategies and precision as larger scale production plants to achieve a reproducible and scalable process (figure 1) [2].

The DASGIP Control* software and associated hardware provides high precision monitoring and control units designed for small working volumes, high information output and easy comparative data analysis. The Eppendorf software DASware® analyze, utilizes the platform-independent Object Linking and Embedding for Process Control (OPC) communication protocol for enabling bidirectional communication between the DASGIP system and third-party analytical devices, including automated bioreactor sampling systems.

Seg-Flow® Automated On-line Sampling System

The Seg-Flow 4800 Automated On-line Sampling System (Seg-Flow System) is a liquid and data management device designed to withdraw samples from up to eight bioreactors and deliver them to up to four analytical instruments and/or fraction collectors. This functionality enables real-time analysis and sample collection from parallel bioreactor systems. The Seg-Flow System's patented "segmented on-line sampling" technology allows a wide range of sample volumes to be obtained and rapidly delivered to distances up to 7.6 meters (25 feet) from the bioreactor.

The FlowWeb™ software platform, which controls all the Seg-Flow System functions, provides seamless connectivity with various third-party analyzers for enabling real-time analysis of important culture process parameters such as nutrients, metabolites and various cell measurements. Upon completion of the analysis, the Seg-Flow system acquires and processes the analyzer data. The FlowWeb OPC software suite communicates the analyzer data into any OPC-enabled supervisory control and data acquisition (SCADA) system, which expands real-time monitoring capabilities for bioprocess cultures. Figure 2 shows the Seg-Flow configuration used by EBI for conducting automated on-line

*DASGIP Control is now DASware control 5. Please refer to ordering information on page 6.

Fig. 2: Seg-Flow 4800 Automated On-line Sampling System with FlowFraction™ 400 Fraction Collector

fraction collection for their microbial strain characterization evaluation.

Process trigger sampling

The Seg-Flow System is capable of performing automated sampling and analysis during planned or unplanned process events in response to an external SCADA or other bioprocess management system such as the DASGIP Control/DASware software platform. This is achieved through OPC connectivity.

The process events used to activate, or trigger, the Seg-Flow System are user-defined. Examples of process events include pH or dissolved oxygen excursions, culture induction, feeding or other in-process control actions. The process events used to trigger the Seg-Flow system require OPC data tag configuration and must be programmed into the host SCADA/bioprocess management system. When the process event is detected by the bioreactor station, the data trigger is communicated to the SCADA system to commence the remote activation of the Seg-Flow system (figure 3).

Once the Seg-Flow system is activated, a sample is

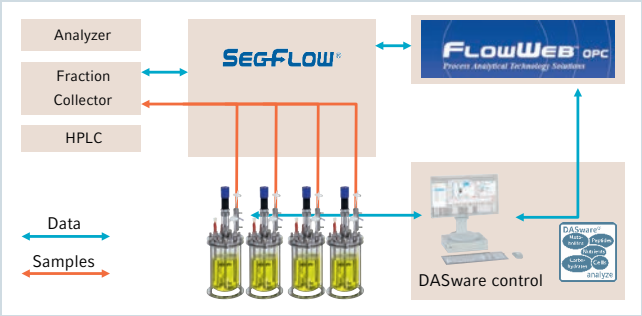

Fig. 3: Architecture for the Seg-Flow 4800 process trigger sampling function. The process event or "trigger" is user-defined and is programmed in the bioreactor's OPC-enabled SCADA or bioprocess management system, which remotely controls the Seg-Flow system.

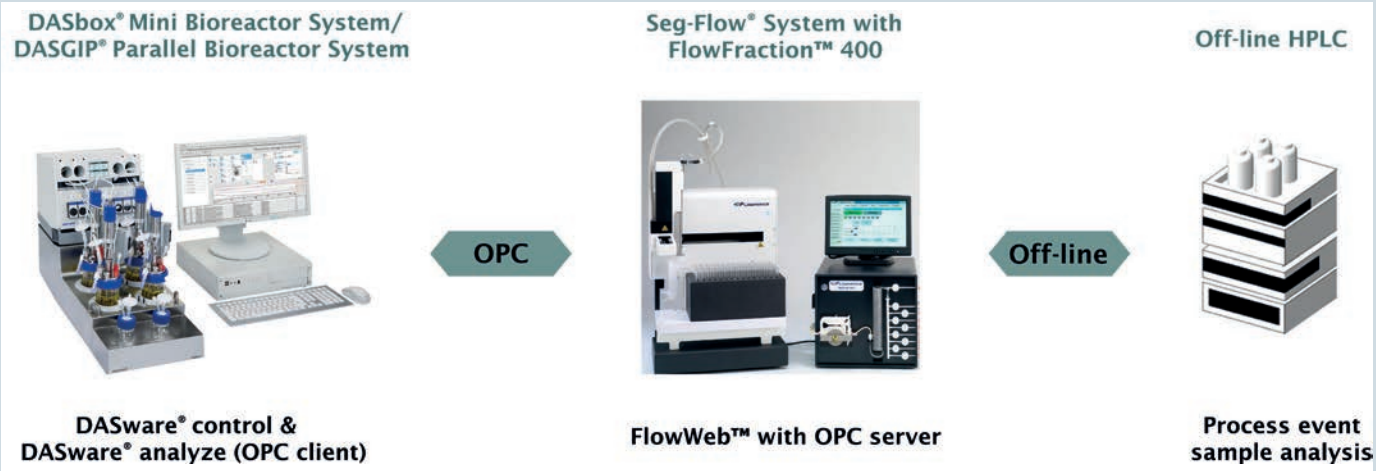

Fig. 4: DASGIP/Seg-Flow Process Trigger Sampling System. OPC communication permits continuous bi-directional communication between the Seg-Flow Automated On-line Sampling System and DASware control. The DASGIP/DASbox system detects the user-defined process event and remotely activates the Seg-Flow System to perform the process trigger sampling function.

automatically withdrawn from the bioreactor for sample collection and/or analysis. Upon completion of the sample collection or analysis, the data is communicated to the SCADA/bioprocess management system via OPC over the laboratory network. When the sampling functions and data transfer are completed, the Seg-Flow System returns to an idle status. The data retrieved from the Seg-Flow system can then be used for additional process monitoring and control options. This unique remote control function allows the process scientist to conduct "around-the-clock" monitoring and sampling of unique process events that could impact process productivity and/or product quality.

Results and Discussion

Integrating the Seg-Flow® and DASGIP® Parallel Bioreactor System

Prior to implementing the Seg-Flow process trigger sampling technology, process events and environmental states affecting yeast stress responses and biofuel production could not be adequately evaluated or characterized due to the lack of automated sampling triggered in response to changing culture conditions.

Using OPC communication, the Seg-Flow automated on-line sampling system was integrated with the DASGIP Parallel Bioreactor System to allow the process trigger sampling technology to be employed (figure 4). Process event tags, which were used to activate the Seg-Flow system for process trigger sampling, were configured and programmed in the DASGIP Control software. The DASware analyze OPC client facilitated OPC connectivity between the FlowWeb OPC server and the DASGIP Control system.

Process trigger sampling

Two yeast cultures were cultivated over a 2.5 day duration using a continuous-culture process. A turbidostat control loop was employed to maintain a prescribed biomass concentration as measured by an *in-situ* optical density probe. The DASGIP Control system activated process media feed and removal from the culture vessels in response to optical density measurements, and user-defined values of media feed volume addition were used as the process trigger events for the Seg-Flow sampling system.

When the desired values of media feed volume addition were reached, the process trigger start command was communicated by the DASGIP Control system to the Seg-Flow system via OPC communication (figure 5). Upon activation, the Seg-Flow system withdrew the programmed sample volume from the bioreactor and delivered the sample to the FlowFraction 400 fraction collector. The collected sample was stored in the fraction collector at a prescribed temperature until the sample was analyzed using an off-line HPLC or other analyzer.

Vessel-specific sample collection data included the beginning and end of the Seg-Flow sample collection phase as well as the sample collection vial position. All data were date- and time-stamped in the FlowWeb software, communicated to the DASGIP Control software using the FlowWeb OPC Server and recorded in the DASGIP Control software. This sample collection data was synchronized in real-time with the fermentation process information and the Seg-Flow Activation time (process trigger time), aligning the remotely controlled sample collection with the process event (figure 5). Also, the remote monitoring functions of the Seg-Flow and DASGIP systems eliminated the need for evening shift coverage and manual sampling.

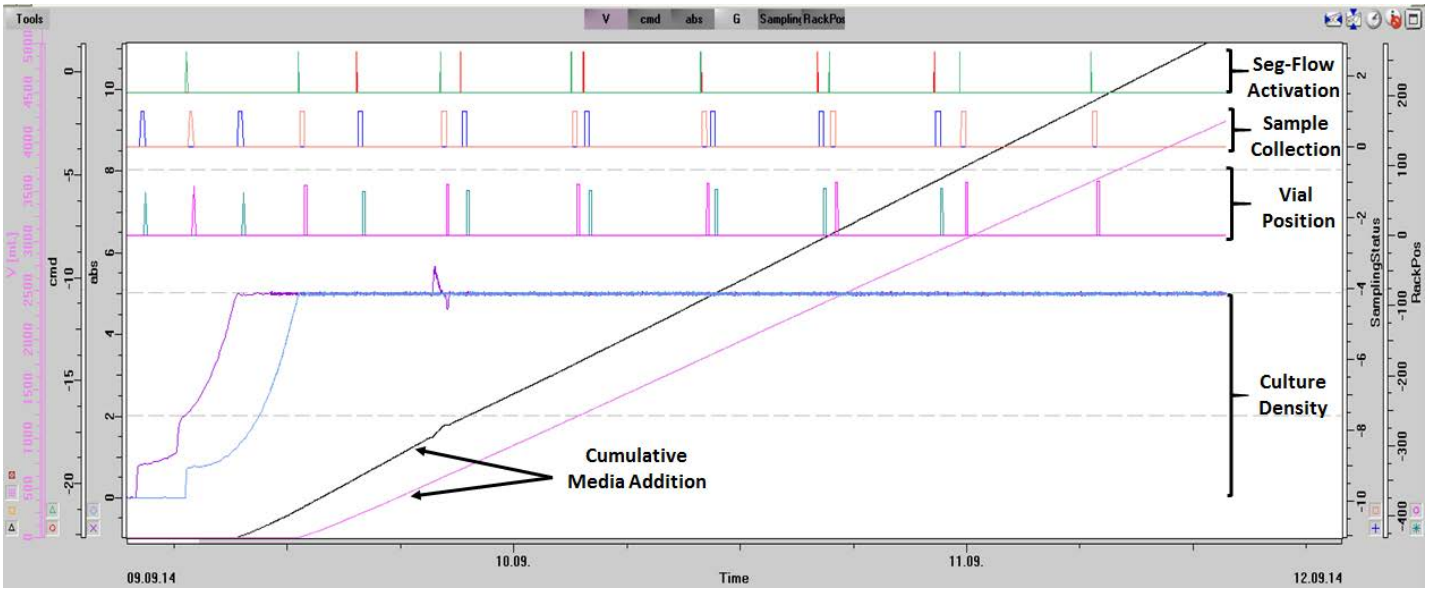

Fig. 5: Process Trigger Sampling Data using DASware Plant Overview Function. Plot displays (**A**) time of Seg-Flow activation by the DASGIP controller (vessel 1 = green, vessel 2 = red); (**B**) time and duration of Seg-Flow sample collection (vessel 1 = orange, vessel 2 = blue); (**C**) time of sample deposition into vial and vial position (vessel 1 = magenta, vessel 2 = green); (**D**) culture density data from biomass probe (vessel 1 = purple, vessel 2 = blue) and (**E**) cumulative media addition (vessel 1 = black, vessel 2 = magenta).

Conclusion

Coupling the DASGIP Parallel Bioreactor and Seg-Flow automated on-line sampling technologies enabled EBI's Microbial Characterization Facility research staff to implement remote-controlled, automated process trigger sampling as an integral part of its yeast strain characterization activities. By integrating this functionality into their high-throughput screening and selection process, EBI research scientists are better able to rapidly characterize process events, parameters and stress responses that impact yeast strain gene regulation and, ultimately, biofuel productivity.

Literature

[1] Energy Biosciences Institute. Biofuels Production Research Programs. Programs and Projects. 24 March 2014. http://www.energybiosciencesinstitute.org/research/biofuels#1.

[2] Knocke, C., Vogt, J. Biofuels - Challenges & Chances: How Biofuel Development can Benefit from Advanced Process Technology. *Eng. Life Sci.* 9-2 (2009): 96-99.

Ordering information

Description	Order no.
DASGIP® Parallel Bioreactor System for Microbial Applications, max. 250 sL/h gassing	
4-fold system with Bioblock	76DG04MBBB
8-fold system with Bioblock	76DG08MBBB
16-fold system with Bioblock	76DG16MBBB
4-fold system, benchtop	76DG04MB
8-fold system, benchtop	76DG08MB
16-fold system, benchtop	76DG16MB
DASware® control, incl. PC, OS, and licenses	
for 4-fold DASGIP® system	76DGCS4
for 8-fold DASGIP® system	76DGCS8
DASware® control professional, incl. PC, OS, and licenses	
for 4-fold DASGIP® system	76DGCSP4
for 8-fold DASGIP® system	76DGCSP8
DASware® analyze, OPC client professional incl. 1x tunneller lic. (OPC DA e.g. for ext. analyzer with autosampler)	
for 4 vessels	76DWANA4P
for 8 vessels	76DWANA8P
for 12 vessels	76DWANA12P

For Information on the Seg-Flow 4800 Automated On-line Sampling System, FlowFraction 400 Fraction Collector and the FlowWeb Software Platform please see www.flownamics.com.

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