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APPLICATION NOTE No. 441

Automated Sampling Using the Bioprocess Autosampler for the Analysis of an *E. coli* Fermentation Process

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Abstract

Bioengineers analyze multiple process parameters at various time points during a bioprocess run, to ensure optimal growth conditions and gain process understanding. While some parameters are routinely measured online, this often also involves the analysis of samples using external analyzers. In this proof-of-concept study, we demonstrate how a DASbox® Mini Bioreactor System was equipped with the Bioprocess Autosampler from Eppendorf. The Bioprocess Autosampler took samples from eight bioreactors which were operated in parallel, at various time points during the fermentation run. The study demonstrates how the use of the Bioprocess Autosampler can increase bioprocess development efficiency by reducing the work of manual sampling.

Introduction

Upstream bioprocess development aims at processes, which deliver the product of interest in a productive manner with consistent quality. To attain this goal, it is critical to understand how the environmental conditions inside the bioreactor influence product formation. Measuring process parameters as well as process performance indicators is an essential prerequisite to successful protocol design. While parameters such as pH, temperature, and DO can be easily followed online, others are often monitored using external analyzers, after taking samples from the bioreactor. Examples include nutrient, product and byproduct concentrations, biomass, and product quality attributes.

During process development, it is appropriate to compare many process conditions and analyze them at multiple time points during the bioprocess run. This strategy requires a substantial number of samples.

The use of autosamplers can increase bioprocess development efficiency by reducing the drudgery of manual sampling while increasing data quality, since the desired sampling interval can be maintained outside normal work hours.

We document in this application note how we equipped a DASbox Mini Bioreactor System with the Bioprocess Autosampler from Eppendorf, which was used for sampling of eight parallel fermentation runs and for storing the samples in a cooling stack. In the first use case, the samples

were used to evaluate protein stability under different storage conditions. In the second use case, the samples were used to analyze the quality of plasmid DNA (pDNA) stored at different temperatures.

Material and Methods

In this study, the Bioprocess Autosampler from Eppendorf was used to take samples from eight bioreactors, which were operated in parallel using a DASbox Mini Bioreactor System. Samples were taken at different time points during the fermentation run.

Configuring the Bioprocess Autosampler

A DASbox Mini Bioreactor System was equipped with the Bioprocess Autosampler (Figure 1). The Bioprocess Autosampler was controlled by DASware control software.

For liquid handling, the Bioprocess Autosampler was equipped with eight tools containing 5000 µL syringes with 23 gauge needles. Their conical style needle tip is optimized for punching through a septum.

To establish the connection between Bioprocess Autosampler and bioreactor, a module was installed, which holds one sample port per reactor. These ports were connected via tubing to the reactor and harbored a septum, which kept the sterile barrier of the bioreactors. For sampling, a needle was automatically inserted into the ports, pinching through the septum.

The samples were transferred to sterile glass vials, which had been placed in a cooling stack.

Sampling strategy

The Bioprocess Autosampler was used to take samples from eight bioreactors, which were run in parallel. During the bioprocess eight samples were taken: After taking an initial sample at the beginning of the run, seven samples were taken in equal time intervalls in the course of the fermentation run time. These intervals were not disrupted over night.

First, a dead volume of 0.8 mL was drawn and discarded. Subsequently, a 4 mL sample was taken and evenly distributed to four glass vials (Figure 2); the aliquots were used for different analyses, including cell density measurements and product analysis. This results in a total sample volume of 39 mL per bioreactor.

Sample count * (sample volume + dead volume) =
8 * (4 mL + 0.8 mL) = 39 mL

The bioprocesses were started with an initial working volume of 200 mL. Removing 39 mL sampling volume resulted in 80.5 % of the initial volume remaining at the end of the run. The samples were transferred to sterile glass vials, which had been placed in sample racks inside the cooling stack. One

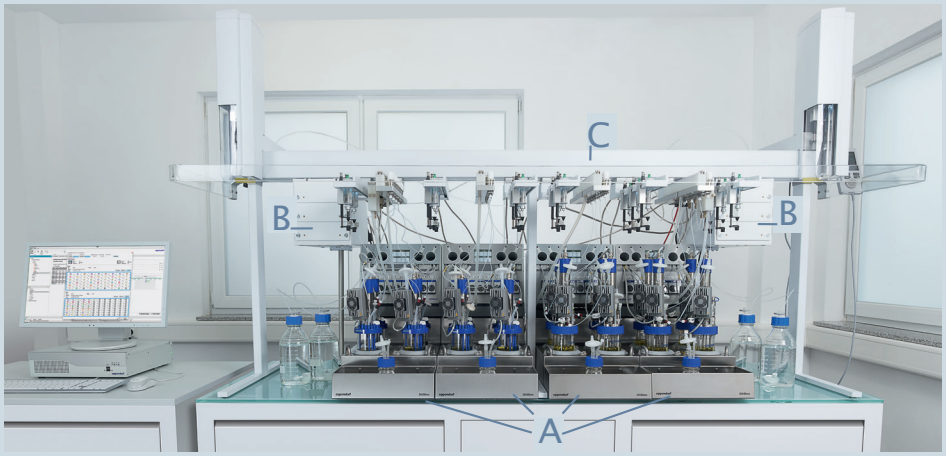

Fig. 1: DASbox Mini Bioreactor System equipped with the Bioprocess Autosampler
A: DASbox Mini Bioreactor System
B: Cooling stack. The cooling stack has three drawers. The temperature of the cooling stack can be regulated from 4 °C to 40 °C.
C: Robotic arm equipped with liquid tools.

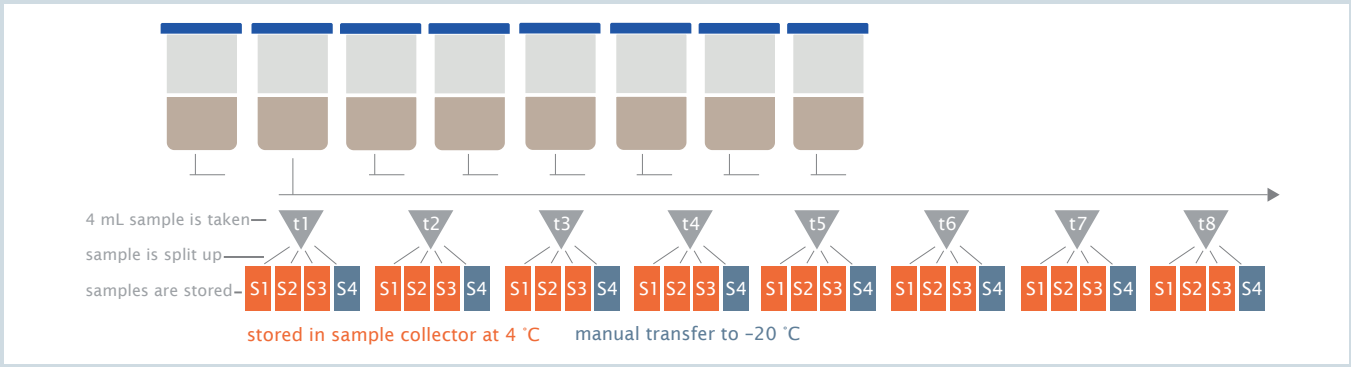

Fig. 2: Sampling strategy. Note text for details

round of sampling all eight bioreactors took 2.5 h. This is including the distribution of the sample to 4 vials. One aim of the study was to compare product storage at 4 °C and -20 °C. The samples for storage at -20 °C were transferred manually to a freezer. To reduce handling errors, samples meant for -20 °C storage were stored in a separate drawer.

Bioprocess parameters

E. coli bioprocesses were carried out in a DASbox Mini Bioreactor System equipped with BioBLU 0.3f Single-Use Bioreactors. The first use case was a bioprocess for protein production. The second use case was a bioprocess for pDNA production. Process parameters for both cases are specified below (Table 1, 2).

Process analytics

Temperature, pH, and DO were monitored and controlled in real-time.

One sample was taken at the beginning of the fermentation run. Starting 6 hours after inoculation, samples were taken every 2.5 hours using the Bioprocess Autosampler. In addition, manual samples were taken, if required, to ensure sampling in the exponential growth phase. Samples were stored at 4 °C or -20 °C for approximately 24 hours and analyzed as follows:

The OD_{600} was measured offline using a photometer. The protein content of samples was analyzed by SDS-PAGE. The whole cell lysates were analyzed with a Coomassie staining and a broad range marker.

Total pDNA and covalently closed circular plasmids (ccc) concentrations were determined after an alkaline lysis with an anion-exchange high performance liquid chromatography (AEX-HPLC).

Table 1: Protein production

Parameter	Configuration
Expression host	*E. coli*
Temperature	37 °C
Culture medium	Complex medium, containing glycerol and animal product-free yeast extract as the carbon and nitrogen sources
Inoculation density	OD_{600} 2-4
Working volume	200 mL
pH	Adjusted to 7.0 with 2 M phosphoric acid and 25 % NH_4OH
DO setpoint	30 %, controlled via adjustment of agitation speed (300 rpm to 2,000 rpm) and gas mix in DO cascade
Foam control	Automatic, by addition of polypropylene glycol
Fermentation duration	Typically 36 h. Fermentation was terminated 30 h after induction
Induction	With 0.25 mM IPTG, induced at OD_{600} = 18

Table 2: pDNA formation

Parameter	Configuration
Expression host	*E. coli*
Temperature	37 °C
Culture medium	Complex medium, containing glycerol and animal product-free yeast extract as the carbon and nitrogen sources
Inoculation density	OD_{600} 2-4
Working volume	200 mL
pH	Adjusted to 7.0 with 2 M phosphoric acid and 25 % NH_4OH
DO setpoint	35 %, controlled via adjustment of agitation speed (300 rpm to 2,000 rpm) and gas mix in DO cascade
Foam control	Automatic, by addition of polypropylene glycol
Fermentation duration	Typically 20 h. Fermentation was terminated when the culture reached stationary growth

Results

Samples were analyzed to determine cell growth, compare product formation at different bioprocess conditions and to evaluate different sample storage temperatures.

Protein production

E. coli cultures were cultivated in an 8-fold parallel DASbox Mini Bioreactor System as described in Material and Methods. Figure 3 illustrates the development of process parameters in a representative fermentation run. Temperature, DO, and pH were monitored online and controlled at setpoint during the experiment. The agitation speed started at the initial setpoint of 300 rpm and increased up to 1200 rpm 9 hours after inoculation and 1.4 hours after induction with 0.25 mM IPTG, reflecting an increasing oxygen consumption of the growing culture.

The OD_{600} and the product were analyzed offline. Samples taken by the Bioprocess Autosampler were divided into four vials and stored at 4 °C and -20 °C. In addition, at t=7.6 h and t=35.7 h it was decided to take samples manually, and store them under the same conditions. While manual sampling is still possible when the Bioprocess Autosampler is in use, it is theoretically also possible to flexibly take additional samples during the fermentation run using the Bioprocess Autosampler, which are outside the schedule defined at initiation of the process.

The OD_{600} reached a maximum of ca. 21 at 35.7 hours.

To evaluate protein stability under the different storage conditions, samples were analyzed by SDS-PAGE (Figure 4). No differences were observed in the band pattern from samples which were taken 3 hours, 7 h, and 21 hours after induction, and subsequently stored at 4 °C or -20 °C. This result establishes that both temperatures were suitable for the storage of these particular protein samples.

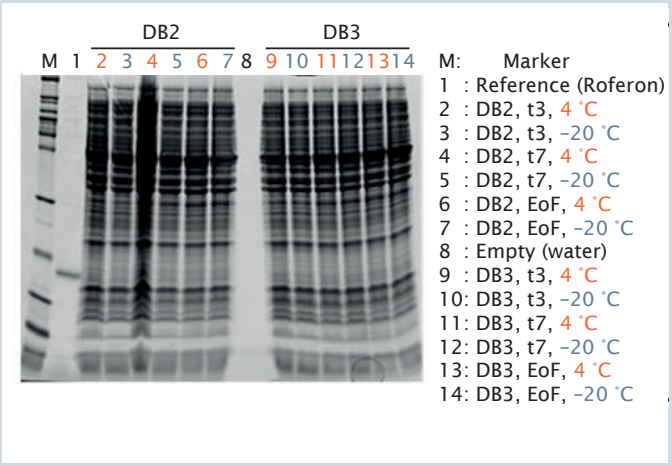

Fig. 4: Protein analysis of samples taken at 3 hours and 7 hours after inoculation (t3, t7), and at the end of the fermentation run (EoF). The samples were stored at 4 °C and -20 °C, respectively. Samples taken from two parallel bioreactors (DB2 and DB3) were compared.

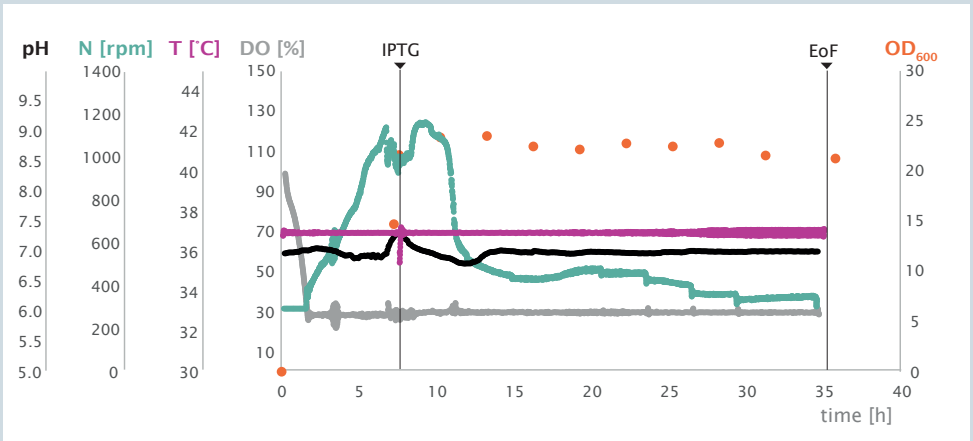

Fig. 3: Process parameters during a fermentation run for protein production. Temperature, agitation speed, DO, and pH were monitored online. The OD_{600} was analyzed offline. For induction, 0.25 mM IPTG was added at 7.6 h, at an OD_{600} of 21.6.

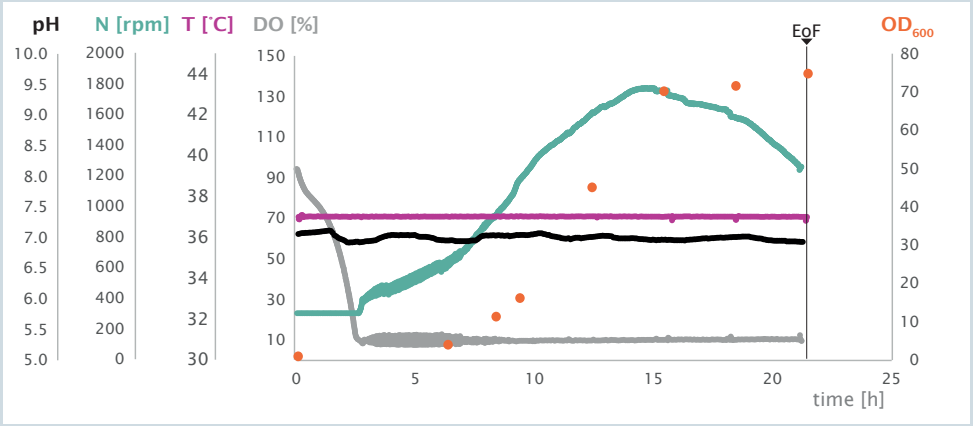

Fig. 5: Process parameters during a fermentation run for pDNA production. Temperature, agitation speed, DO, and pH were monitored online. The OD_{600} was analyzed in samples taken by the Bioprocess Autosampler with the exception of the sample at the end of fermentation, which was taken manually.

pDNA formation

E. coli cultures were cultivated in an 8-fold parallel DASbox Mini Bioreactor System as described in Material and Methods. Figure 5 illustrates the development of process parameters in a representative fermentation run. Temperature, DO, and pH were monitored online and controlled at setpoint during the fermentation run. The agitation speed (N) started at the initial setpoint of 300 rpm and increased up to 2000 rpm approximately 14 hours after inoculation, reflecting an increasing oxygen consumption of the growing culture.

The OD_{600} and the product were analyzed offline. The OD_{600} reached a maximum of ca. 72 at the end of the fermentation run (EoF).

Samples were stored either at 4 °C or -20 °C. The pDNA concentration in samples stored at -20 °C was lower than in samples stored at 4 °C. In contrast, the proportion of covalently closed circular plasmids (ccc) was lower in samples stored at 4 °C (Figure 6). The lower porportion of ccc plasmids suggests that a higher percentage of plasmids exists in open circular and linear forms. These more open forms are caused by reversible strand nicks of the plasmid. Thus, storage at -20 °C is probably beneficial for the homogenity of the plasmid sample.

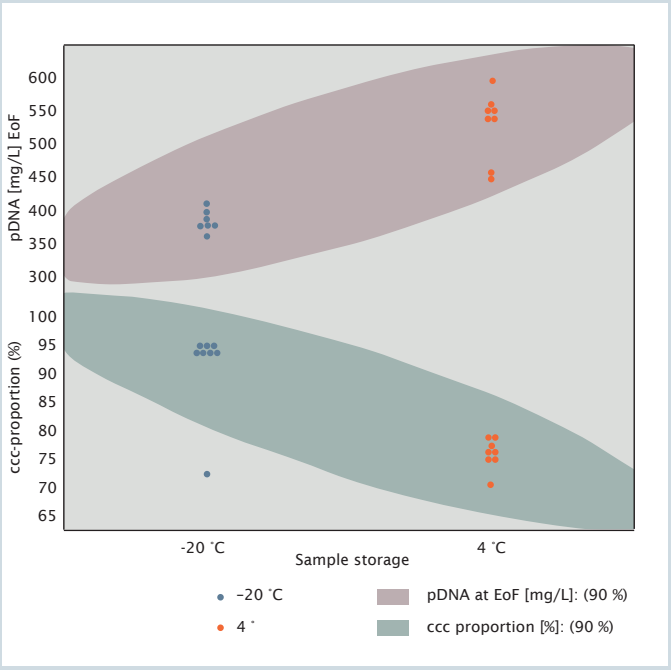

Fig. 6: Analysis of pDNA in samples stored at 4 °C and -20 °C, respectively. ccc: Covalently closed circular plasmids

Conclusion

In the study presented here, an 8-fold parallel DASbox Mini Bioreactor System was equipped with the Bioprocess Autosampler from Eppendorf. The sampler needed only little additional space, as it was mounted on the lab bench above the bioreactor system. Sampling was performed under aseptic conditions with local sterility, comparable to the conditions when sampling manually.

Typically eight fermentation runs were carried out in parallel and eight samples per bioreactor were taken during each fermentation run. Thus, 64 sampling steps were performed by the Bioprocess Autosampler, which drastically reduced the routine manual workload. Sampling was independent from normal working hours which ensured high consistency and avoided gaps in the collection during the night and at the weekend. For high flexibility, samples can be removed from the cooling stack at any time, for example to store them at lower temperatures as described in this study. The time needed for automatic sampling from eight bioreactors in the described setup was 2.5 hours. While a 2.5-hour sampling interval is suitable for many fermentation applications, shorter intervals may be desirable, especially to characterize a newly established process. To shorten the sampling intervals, splitting samples to multiple vials, as in the study described here, can be avoided. Additionally, a dual-head version of the Bioprocess Autosampler is availabe to further increase the sampling efficiency to up to more than one sample per hour per bioreactor. The data presented here establish the convenience and versatility of the combined use of the DASbox Mini Bioreactor System and the Bioprocess Autosampler and their performance as an integrated unit.

The control of the Bioprocess Autosampler is seamlessly integrated with DASware control 6 software, allowing flexible and customizable sampling schemes, like pre-setting samples prior to starting the experiment, scheduling additional samples outside the initial scheme in case of special events, and including manual samples in the storage pattern.

Ordering information

Description	Order no.
DASbox® Mini Bioreactor System, for microbial applications, max. 25 sL/h gassing, 4-fold system	76DX04MB
DASware® control, including PC, OS, and licenses, for 4-fold DASbox® Mini Bioreactor System	76DXCS4
BioBLU® 0.3f Single-Use Bioreactor, fermentation, open pipe, 2 Rushton-type impellers, no pH, sterile, 4 pieces	1386100100
Bioprocess Autosampler	inquire

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