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White Paper

Automated Aseptic Sampling for Accelerated Access to Process and Quality Data in Upstream Bioprocessing

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The biopharmaceutical market is experiencing increased demand for new medicines, reduction of costs, and new product classes, which is driving the need for increased flexibility and cost control measures in manufacturing. The concept of Biopharma 4.0 seeks to accomplish these targets through several strategies, including implementation of process analytical technologies (PAT) and integration of digital technologies and new data management tools.

This white paper focuses on PAT and the use of automated sampling technology to accelerate analytical and quality control methods and provide an approach for access to in-line data to monitor processes in real time.

PAT and Automated Sampling

The application of PAT requires analytical monitoring, data analysis, process control, and continuous optimization to be successful. Process monitoring leverages technologies such as sensors, sampling, and analytical methods to measure critical process parameters (CPPs) and critical quality attributes (CQAs). Once measurements are made, mechanistic or data-driven modeling is applied to better understand the data that have been generated. With an understanding of the process, control and automation can be used to improve the process and potentially improve the quality or efficacy of the product. Finally, continuous process optimization and knowledge management are applied to gain further improvements in a Quality by Design (QbD) approach.

Automated sampling is an enabling technology that offers the ability to automate process analytics with online data acquisition. Several process steps can benefit from this type of automation, but it has most commonly been applied in upstream bioprocessing for improvement or simplification of the bioreactor process. With a manual approach, samples must be taken periodically from the bioreactor by an operator to measure and monitor the process. Some at-line analyses may be performed, and the remainder of the sample will be saved and stored for off-line analyses at a later time. This approach is quite labor- and time-intensive. In contrast, an automated workflow eliminates the points of manual intervention that require an operator to be physically present.

With an automated sampling system, samples can be pulled from the process stream and sent to different analyzers to obtain data without the need for an operator. This can replace both at-line and off-line sample analysis because a variety of analyzers can be connected to the sampling system. Automated sampling expands the capabilities for on-line measurement of CPPs and CQAs compared to traditional in-line sensors (pH, dissolved oxygen, biomass) or even multi-attribute sensors such as those utilizing Raman spectroscopy (Table 1). When used in combination, automated sampling can help reduce the manual burden for calibration or model development of in-line sensors.

	In-Line		On-Line
	Traditional Sensors	Raman	Automated Sampling
Temperature	✓		
Pressure	✓		
Conductivity	✓		✓
Gases (DO, CO_2)	✓		✓
pH	✓		✓
Cell density/biomass	✓	✓	✓
Titer		✓	✓
Aggregation		✓	✓
Nutrients/Metabolites		✓	✓
Vitamins & amino acids		✓	✓
Glycans		✓	✓
HCP			✓
DNA			✓
Charge Profile			✓
Fragmentation			✓

Reduce manual burden and improve accuracy with in-line technologies through automated calibration & model development.

Integrate with a broad range of analyzers to enable automated measurement of parameters that cannot yet be measured in-line.

Table 1.

Automated sampling expands capabilities for measurement of CPPs and CQAs in combination with in-line sensors.

Figure 1 provides a schematic of the process set-up for the experiments described in this white paper and shows that automated sampling can be integrated into an upstream bioprocess with other PAT tools. In this process, a 3 L stirred tank bioreactor was run in perfusion mode utilizing a cell retention device to remove spent cell culture medium and replace it with fresh medium. An automated sampling system was connected to the bioreactor to pull samples automatically for on-line analysis. In parallel, a Raman analyzer was inserted into the head plate of the bioreactor for continuous in-line measurements.

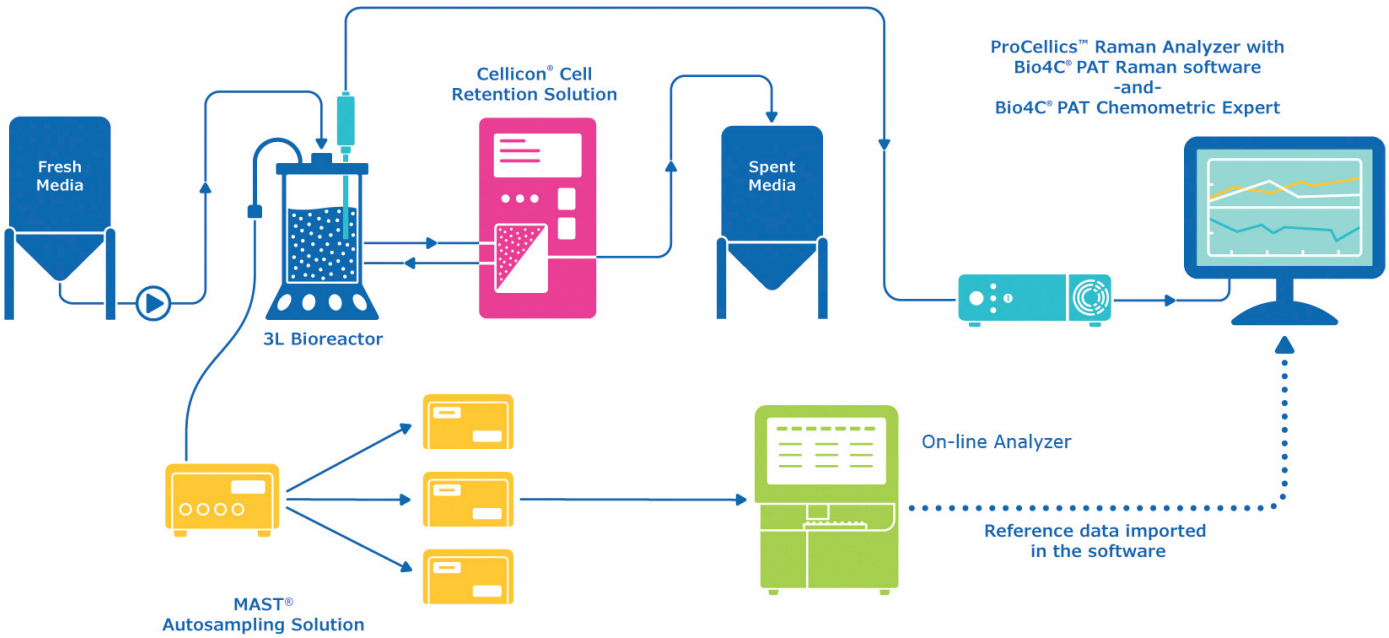

Figure 1.

Automated sampling can be integrated with other PAT tools in the upstream bioprocess.

Benefits of Automated Sampling in Upstream Process Development

We have estimated that a typical perfusion bioreactor run utilizes approximately eight hours of labor spent on sample acquisition and at-line analysis, oftentimes requiring operators be onsite during weekends and at off-hours. During process development, when many conditions are run in parallel, the sampling and analysis workload can quickly add up. This limits the potential output from a set of experiments, either through a maximum number of conditions that can be tested at once or through less frequent access to data. Additionally, samples that are sent for off-line analysis can require weeks to be returned. For these reasons, experimental results are often less complete, robust, or timely than desired and a lack of information can then lead to incorrect decisions or wasted time during process development.

To address these challenges, the MAST® Autosampling Solution was installed to automate aseptic sampling and sample management (Figure 2). The system allows sampling from five points (i.e., bioreactors) and sample delivery to four primary analyzers. With this system, bioreactor sterility can be maintained while improving process understanding with real-time and accurate analytical results.

Figure 2.

MAST® system for automation of aseptic sampling and sample management.

Following installation, a proof-of-concept study was performed to evaluate the ability of the MAST® Autosampling Solution to maintain sterility of the cell culture and accuracy of the sample data. A set of three perfusion runs were performed; two were performed in steady state perfusion mode at 80 × 10^6 cells/mL for approximately 16 days and the third was performed in N-1 perfusion mode in which the cells grew to a density of about 150 × 10^6 cells/mL. The high-cell density run was used to challenge the system and ensure that at higher densities, with higher viscosities, the system could still measure the samples effectively. Over the course of these runs, the MAST® Autosampling Solution took samples four times a day or every six hours.

Once a day, a manual sample was taken within ten minutes of one of the automated samples to assess accuracy of the data between the automated and manual samples.

As shown in Figure 3, the desired level of cell growth was achieved across all three runs and there was no contamination detected, indicating that sterility of the system was maintained. At the high cell density, there were no difficulties with the MAST® Autosampling Solution to deliver cell culture to the destination or analyze the samples. When comparing the automated sample data to the manual sample data taken once a day, across a variety of analytes, there was good correlation as indicated by results falling close to the y = x line.

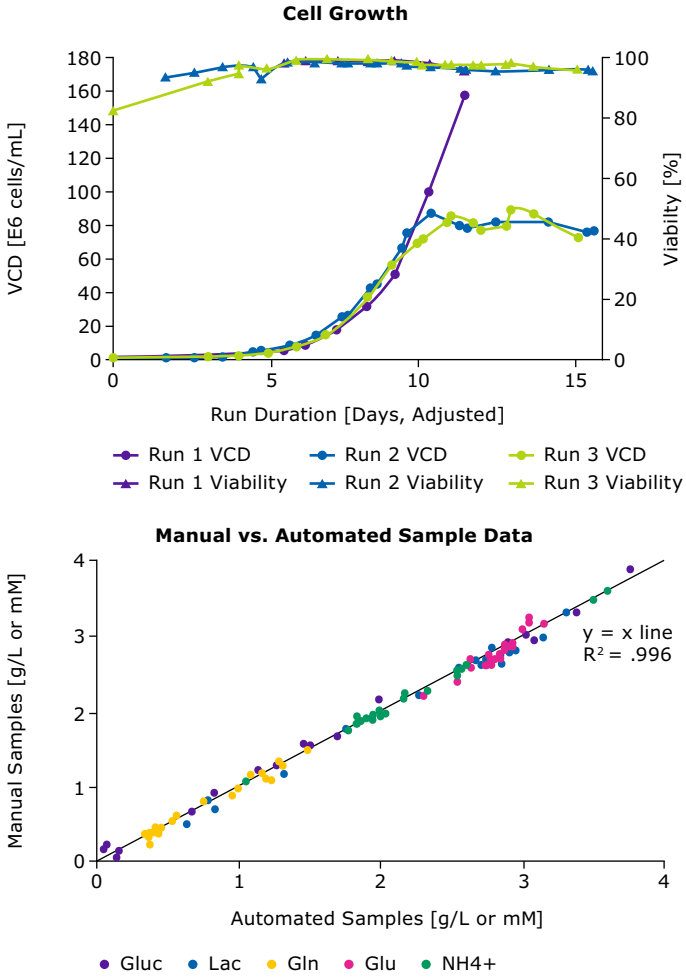

Figure 3.

Automated sampling proof of concept was used to verify sterility and accuracy of sample data.

The next objective was to understand the benefits of implementing automated sampling in perfusion process development experiments. These runs were performed in a 3 L glass bioreactor with a 2.2 L working volume in steady state perfusion mode with a target cell density of 80 × 10^6 cells/mL. Cell health was maintained with a constant perfusion rate of 20 pL/cell/day, or approximately 1.5 vessel volumes per day, using an EX-CELL® Advanced HD Perfusion medium. Manual samples were taken daily, as per normal protocol, which required about eight hours of operator time including weekend work. Additionally, samples were sent to an offline lab for analysis of product quality, which resulted in a six-week turnaround time.

In one of these process development experiments, the MAST® Autosampling Solution was implemented and connected to a Nova BioProfile® FLEX2 for measurement of nutrients, metabolites, cell density, osmolarity, pH, and gases. Figure 4 shows the resulting data for glucose, viability, ammonium, and lactate, as compared to the daily manual sample data, indicating similar trends regardless of the method. Use of automated sampling provided an increase in the frequency of data acquisition with much less effort; four times more data were collected, and the frequency could have been increased even further. The only resources needed for analysis of the automated samples was a 20-minute setup procedure and periodic cleaning procedures as initiated by the operator, eliminating eight hours of time spent on sample acquisition and analysis in a manual run. Additionally, the increased frequency of data allowed for improved comparison between conditions across other process development runs and improved the decision making capabilities of the team.

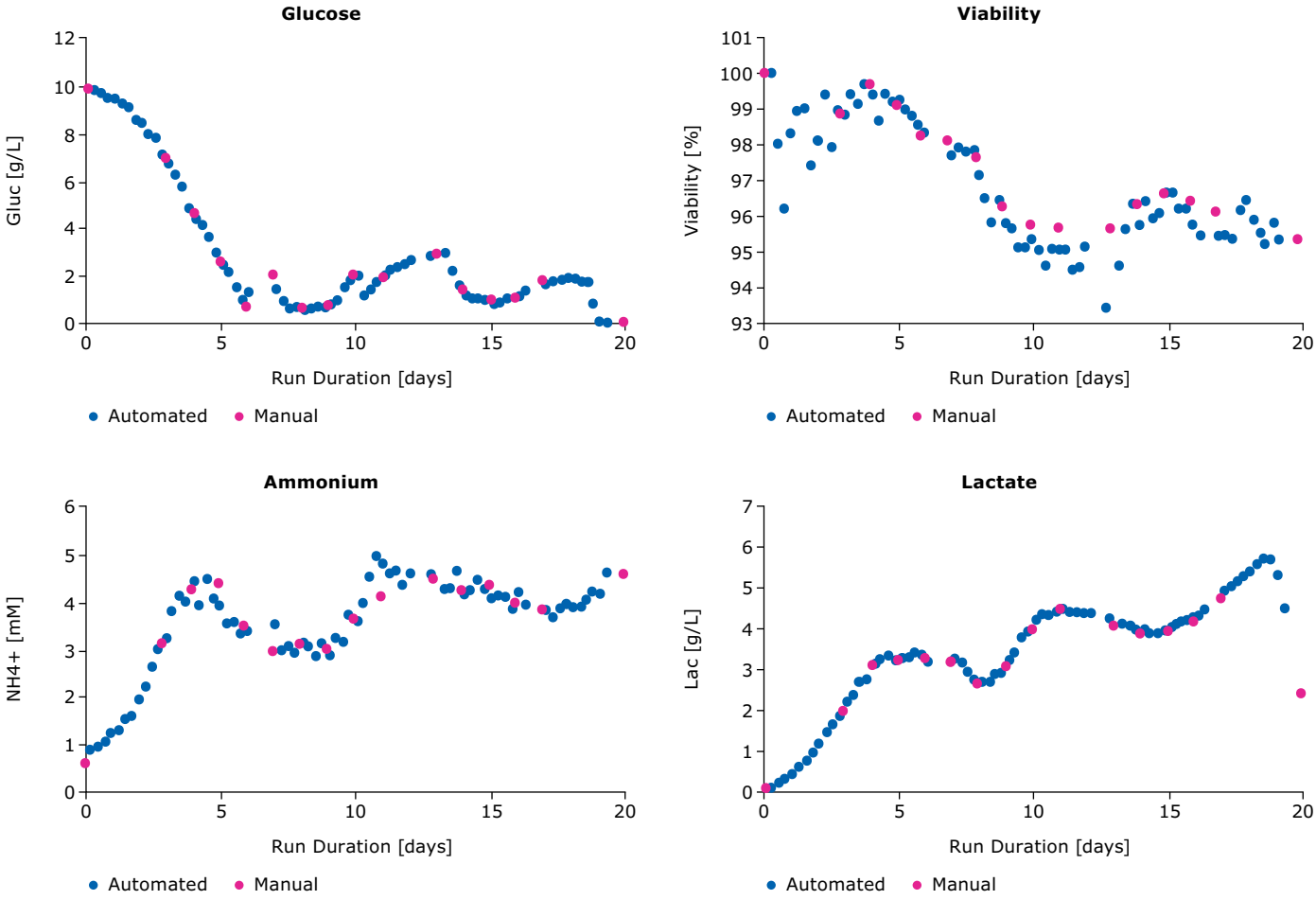

Figure 4.

Automated sampling increases the frequency of data acquisition with less effort.

The robustness of the MAST® Autosampling Solution was also assessed across a set of process development runs. Three different bioreactors were run in parallel with 171 total samples scheduled. Only five samples were missed, providing a 97% successful completion rate and meeting the expectations of the team. Some missed samples were caused by a clog within the analytical instrument and could have been mitigated with more regular cleaning procedures. Others were caused by the instrument being out of calibration or lacking appropriate reagents, which is also preventable with regular maintenance.

Combination of PAT Tools

As noted above, automated sampling can supplement other PAT tools for reduced effort and improved results. Figure 5 shows an experiment in which automated sampling was used together with an in-line Raman spectroscopy probe, the ProCellics™ Raman analyzer with Bio4C® PAT Raman Software. After a chemo metric model was developed N-1 perfusion cell cultures, data was monitored in-line to assess transferability to dynamic perfusion cultures. The results showed that the in-line Raman sensor data was comparable to automated sampling on-line data with the MAST® Autosampling Solution. The automated sampling data could be used to validate this model and pick up on deviations if they had occurred. Additionally, when implemented in the model development phase, automated sampling has significantly reduced the workload to gather sample data. This can lead to shorter model development timelines with fewer replicates needed to obtain the same number of samples, and improved robustness by capturing the full scope of process variability with more frequent samples.

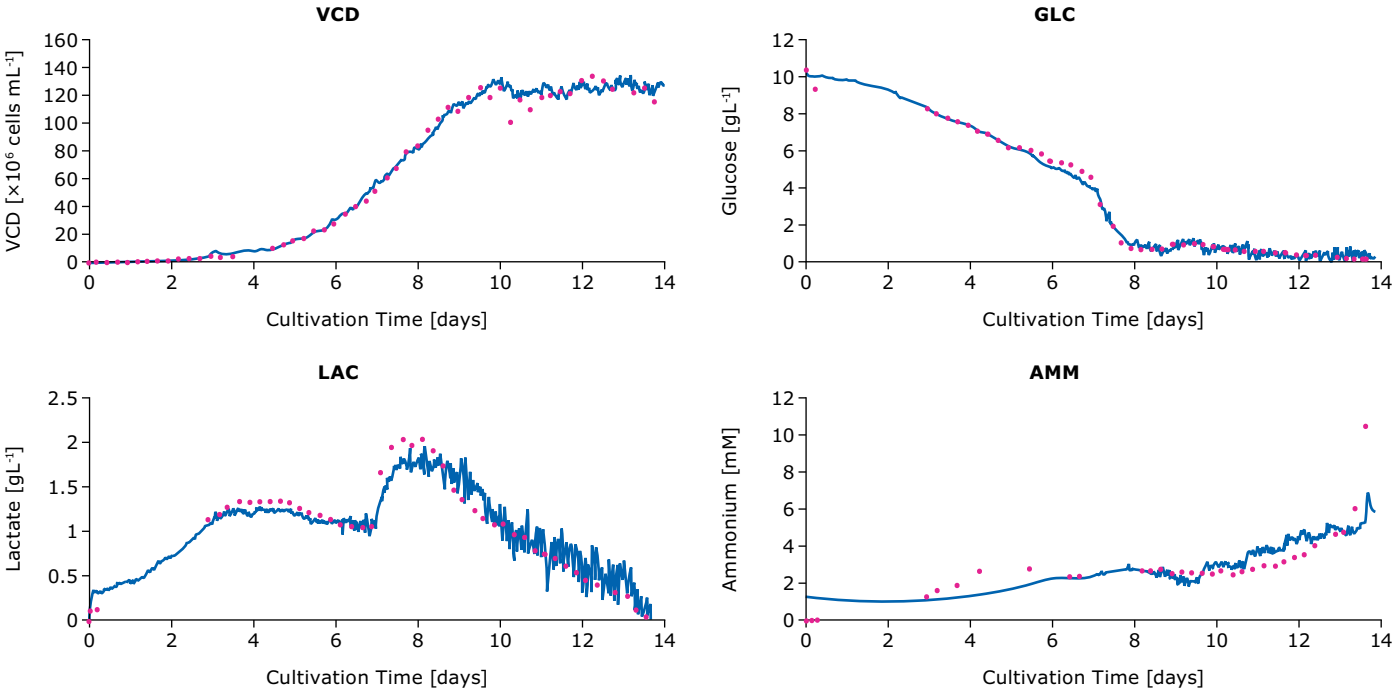

Figure 5.

Automated sampling can supplement other PAT tools such as Raman.

Conclusion

These experiments describe the initial steps towards obtaining online data from a bioprocess, demonstrating the value of doing so in an upstream process development lab. Implementation of automated sampling helps alleviate the burden on manual sampling from the bioreactor while providing improved data sets for quicker and more effective decision-making.

Automated sampling fits an industry need to acquire online sample data and can be used in combination with other PAT tools for optimal results. Upcoming projects aim to connect additional analyzers with the MAST® Autosampling Solution to increase the range of CPPs and CQAs that can be measured on-line with automated sampling.

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