On-line Measurement of Glucose and Lactate with Automated Feedback Control

MAVEN™ provides continuous, on-line monitoring with measurements as often as every two minutes, with no loss of bioreactor volume and no risk of contamination.

Introduction

The past decade has seen enormous growth in demand for biotherapeutic drugs—complex molecules that can only be produced by living cells. With expansion of modalities such as gene and cell therapies, the trajectory for biotherapeutics has never been higher. In the race to develop efficient production methods for these novel drugs, one significant bottleneck remains: monitoring bioprocesses in real-time to control critical process parameters (CPPs) and ensure product quality.

Enter the MAVEN, an on-line device with an *in-situ*, autoclavable probe that measures glucose and lactate without consuming any bioreactor volume, thus eliminating the need for manual sampling. Glucose (cells' primary energy source) and lactate (a byproduct of metabolism) are the most requested and critical biochemical parameters measured during a bioprocess. Integrated PID control allows users to connect a pump for automated feeding.

Figure 1 shows the configuration of MAVEN, which can be used in a wide variety of bioreactor types and sizes across several applications in cell culture and fermentation. In addition to the *in-situ* probe, MAVEN can be connected to a perfusion bioreactor through a diffusion flow cell.

MAVEN answers the urgent need for on-line measurements and automated control of glucose levels, which reduces manual labor, lowers risk of contamination from sampling, decreases risk of human error with feeding, and reduces the stress that cells may experience from variable glucose levels. Ultimately,

Figure 1. MAVEN components and connection to a 2L, single-walled bioreactor from Sartorius

- **Diffusion probe can be inserted into the PG13.5 port of the culture vessel.**
- **The diffusion membrane within the probe only allows small molecules such as glucose and lactate to enter. Membrane and probe are both autoclavable to prevent chance of contamination. These analytes are transported by buffer solution to the biosensor.**
- **Calibration standard ensures that the biosensor continues to operate within expected parameters.**

MAVEN helps ensure optimal productivity and consistent product quality.

Here we share the methodology to monitor and control glucose levels in real-time using MAVEN, plus the resulting data.

Experimental Design

On-line Measurement of Glucose & Lactate Using MAVEN with Continuous Control of Glucose at 1 and 2 g/L

Monoclonal antibody-expressing GS-CHO cells were cultured in three Biostat® 10L bioreactors (Sartorius Biostat® B-DCU II with BioPAT DCU Tower) in a fed-batch process using chemically defined CD FortiCHO™ media and CD EfficientFeed™ C (Thermo Fisher Scientific). Cells were seeded at 0.3 million cells /mL in 7.9L of media and grown at 37°C. The dissolved oxygen set point was 30% and the pH set point 6.9 with deadband of 0.03. EfficientFeed C was fed every other day at 4% v/v starting on day 3.

• On-line measurements of glucose and lactate were taken every 20 minutes using the stainless-steel MAVEN diffusion probe directly fitted into the bioreactors.

• Off-line measurements of glucose and lactate were taken using a Cedex Bio HT Analyzer (Roche) daily to confirm measurements from MAVEN.

• Automated glucose continuous feeding was delivered by a feed pump controlled by MAVEN at 1 or 2 g/L setpoints (Bioreactor A and B, respectively) as shown in Table 1. The integrated automatic glucose feeding provides a hands-off method to achieve continuous glucose levels that may minimize stress on the cells and remove the need for manual feeding.

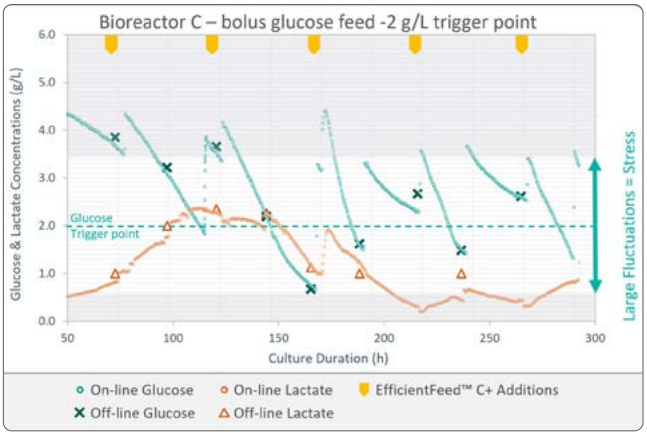

Figure 2. On-line (MAVEN) and off-line glucose and lactate measurements during 14-day time course in bioreactor C—our control bioreactor with default, bolus feeding strategy of trigger point 2 g/L, add up to 4 g/L. The area highlighted in white shows the fluctuation of glucose levels caused by the glucose bolus feeding during the cell culture duration.

• Manual bolus feeding strategy (Bioreactor C): glucose was fed at 4g/L when off-line measurement fell below the 2g/L trigger point (2–4 g/L).

Results

In all three bioreactors, the MAVEN on-line glucose and lactate measurements, collected every 20 minutes, were well aligned with the daily off-line readings obtained with the CEDEX BioHT analyzers as shown:

• Figure 2 for bioreactor C, our control bioreactor with bolus feeding, as usually done at the CPI NBMC,

• Figure 3A for bioreactor A with low-level continuous glucose feed (1g/L setpoint), and

• Figure 3B for bioreactor B with medium-level continuous glucose feed (2g/L setpoint).

The lactate profiles show the traditional shift from lactate production to lactate consumption, which is indicative of a healthy productive CHO cell culture.

Bioreactor name	Glucose feeding	Glucose level	Glucose control
A	Continuous	1g/L setpoint	MAVEN
B	Continuous	2g/L setpoint	MAVEN
C	Bolus	2g/L trigger point (add to reach 4 g/L)	Manual addition

Table 1. Bioreactor set-up with glucose feeding parameters. Glucose and lactate measurements were performed off-line with Cedex Bio HT Analyzer (Roche) and on-line with MAVEN (908 Devices).

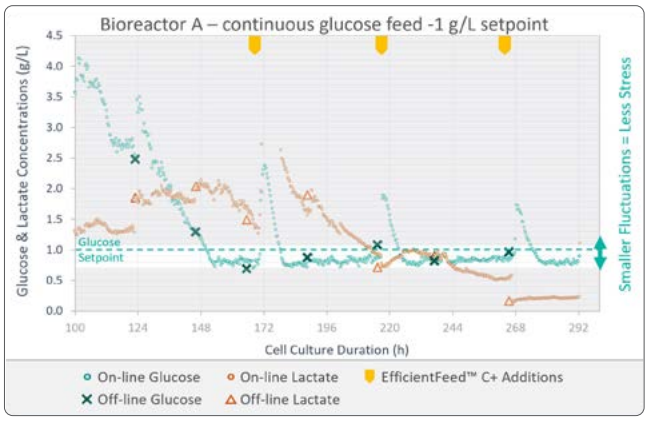

Figure 3A. On-line (MAVEN) and off-line glucose and lactate measurements in bioreactor A with cells automatically fed using the MAVEN for continuous glucose at setpoint 1g/L.

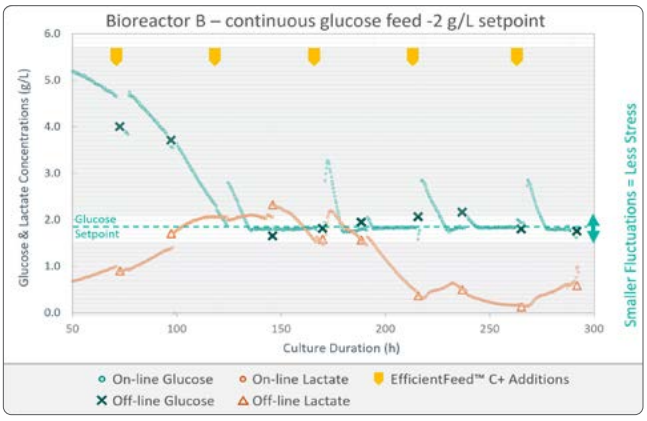

Figure 3B. On-line and off-line glucose and lactate measurements in bioreactor B with cells automatically fed using the MAVEN for continuous glucose at setpoint 2g/L.

The area highlighted in white shows the fluctuation of glucose levels outside of the EfficientFeedC + additions in the cell culture. The variation of glucose levels outside the feed spikes is remarkably low, showcasing the ability of MAVEN to deliver precise control of glucose, thus reducing cell stress and the risk of cell culture crashing.

In bioreactors A and B, glucose was fed continuously using the MAVEN integrated PID controller, as shown in Figures 3A and 3B. As expected, the glucose levels were maintained near the glucose set point of 1 g/L or 2 g/L, respectively, enabling a tight control of the glucose concentrations in the bioreactors without large variations. The spikes in the glucose levels are caused by the addition of the commercial feed (containing glucose).

As shown in Figure 4, cell growth and viability in Bioreactor A (low level, 1g/L continuous glucose feed) were comparable to Bioreactor C (control bioreactor with default 2–4 g/L bolus feeding strategy). Cell growth was not affected by the different feeding strategies, and cell viability remained high until the end of the cell culture, even with a low level of glucose available.

Conclusion

MAVEN ultimately provides peace of mind when developing a bioprocess. The system's continuous on-line monitoring of glucose and lactate, along with hands-off feed control of glucose levels significantly reduce the risk of human error, contamination, and glucose level fluctuations that can cause cells stress in bioprocess. With flexible integration options for PD and GMP, MAVEN is easy to set up, operate, and maintain.

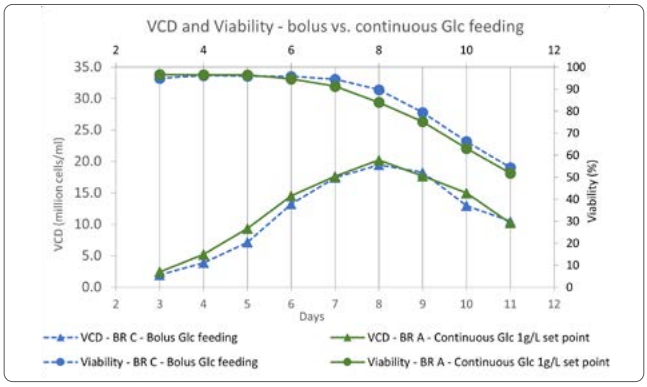

Figure 4. Comparison of cell growth (viable cell density, VCD) and cell viability between control bioreactor C—bolus feeding, and bioreactor A, MAVEN -controlled automated continuous glucose feeding with 1 g/L setpoint. The cell cultures grew and sustained in a remarkably similar way, showcasing how low levels of glucose do not hinder cell growth or reduce viability.

The 20-minute measurement interval was optimal for mammalian cells in our fed-batch bioprocess. However, more frequent measurements, down to every two minutes, are available for faster process cycles, or for less robust processes such as those for gene and cell therapies.

This work was performed at CPI's National Biologics Manufacturing Centre (NBMC), Darlington, UK.