POSTER APP NOTE

Enabling Real-time Process Control

Automated Glucose Feeding Based on Online Glucose and Lactate Measurements

Highlights

• Automated and continuous glucose and lactate monitoring of your bioprocess with the MAVEN on-line device with an *in situ* probe

• Enabling accurate control of glucose levels for improved growth, productivity, and product quality in cell and microbial cultures

• Sensitive, selective, precise and robust measurements with biosensor technology

• Sampling through semipermeable membrane probe *in situ*: no bioreactor/ fermenter volume loss upon sampling – no contamination risk introduced with sampling

• Solution for **fed-batch** and **perfusion** cell and microbial culture monitoring

Overview

Reduced development timelines, process intensification, and automation of bioprocess controls are key manufacturing initiatives. Yet, manual handling of samples is still common for time-sensitive, critical bioprocess parameters.

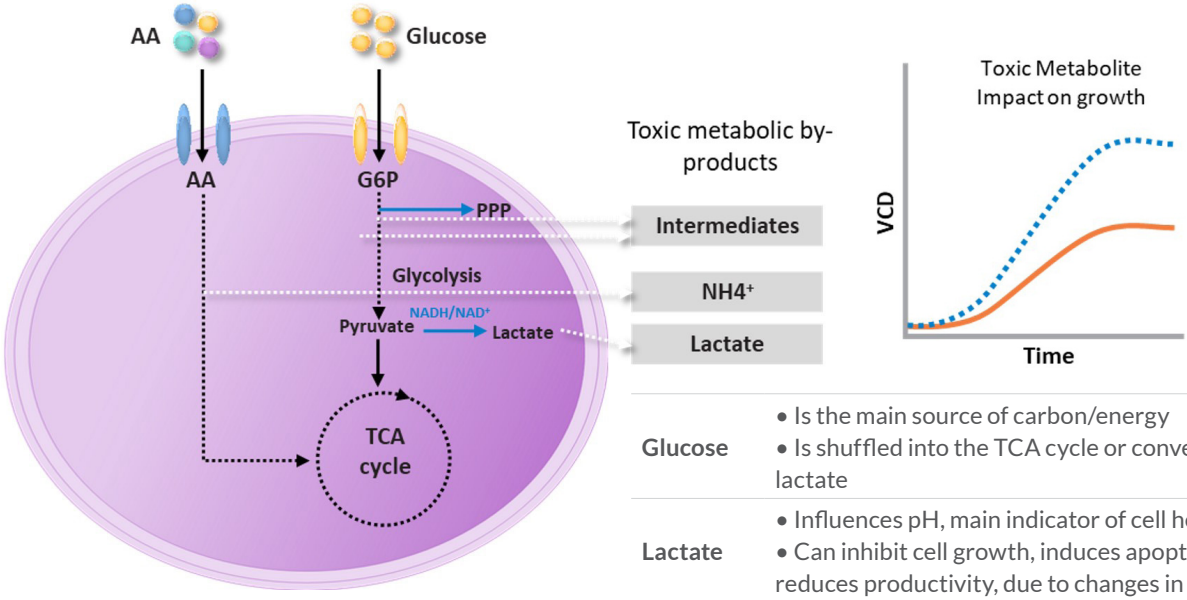
The ability to adjust glucose feed rate benefits cell growth and viability. Lactate is a primary metabolite in cell culture. Controlling glucose and lactate levels leads to more consistent drug substance quality and yield.

Defined control strategies enable more intensified and stable cell culture processes such as perfusion which may see dramatically improved productivity. This results in a more efficient and profitable process for biotherapeutics manufacturing.

In this study, we describe an automated control strategy for production of biologics. It leverages the MAVEN, a reliable and easy-to-operate, on-line (sample-free) device for monitoring of glucose and lactate with automated glucose feed control.

Real-time Monitoring of Glucose and Lactate and Glucose Control

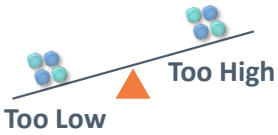
Glucose and Lactate Measurements in Bioprocess



Glucose	• Is the main source of carbon/energy • Is shuffled into the TCA cycle or converted into lactate
Lactate	• Influences pH, main indicator of cell health • Can inhibit cell growth, induces apoptosis and reduces productivity, due to changes in pH and osmolality

Figure 1: (Modified from Ref 1) Glucose and lactate are key players in cellular metabolism as energy source and by-products. A clear understanding of cell requirements is critical in process development and controlling these essential parameters in manufacturing.

How Much Glucose & Lactate Do Cells Need?

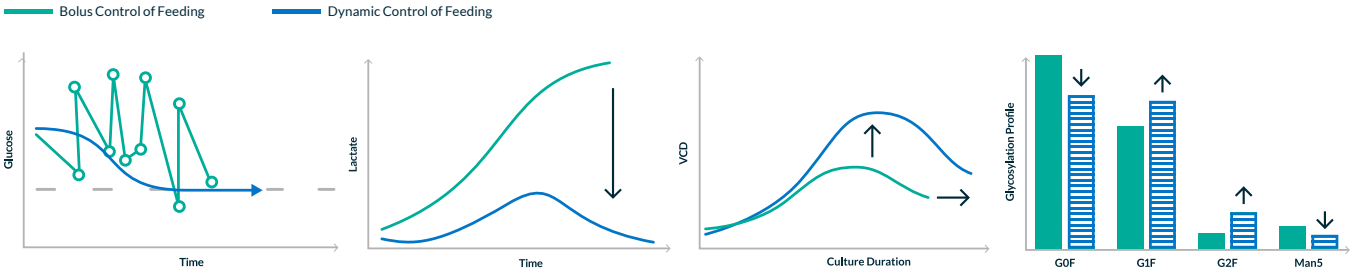
Glucose	Lactate
	• **Inhibited** cell growth • **Reduced** productivity • **Reduced** product quality • **Lower** antitumor activity
	• **Higher** maximum viability • **Increased** product yield • **Better** product quality • **Robust** process

Just the right amount at the right time.

*"A small **difference in the timing** of glucose feeding may cause cultures with very similar metabolic behavior to **diverge to different outcomes**"*

Table 1: A summary of glucose and lactate contributions to overall health of cell culture in bioprocess.

Benefits of Automated vs Manual Feeding



Maintain lower range of glucose concentration and minimize nutrient fluctuations

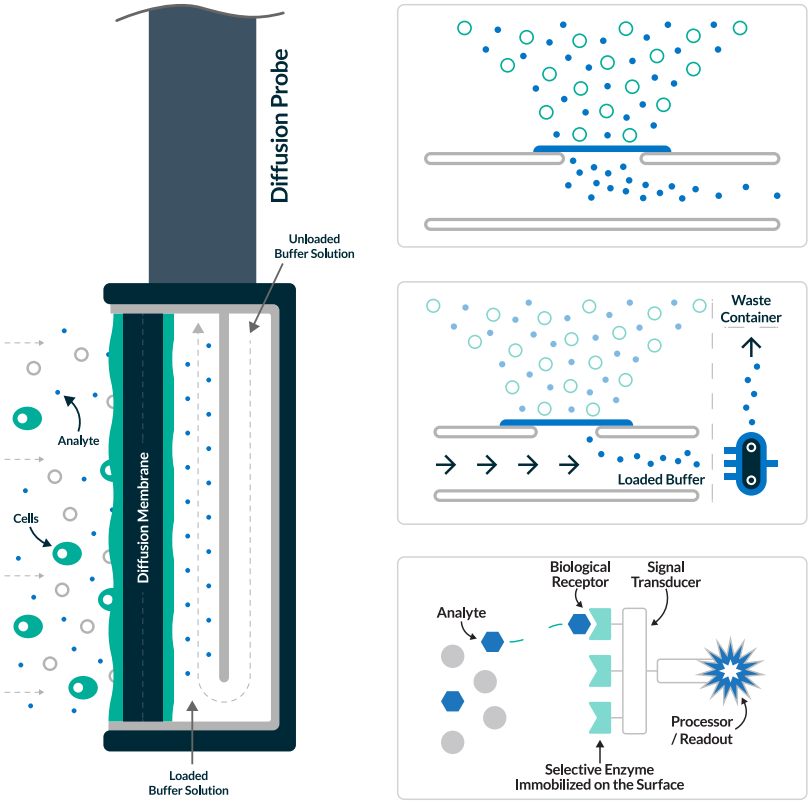
Optimize lactate metabolism and prevent growth-inhibiting lactate accumulation

Improve cell growth, increase maximum viable cell density, and total product yield

Improve product quality such as glycosylation profile and reduce unwanted glycation

Figure 2: Tight control of glucose-lactate concentrations enables development an implementation of dynamic feeding strategies to optimize cellular metabolisms leading to higher prodcutivity and improved quality of biotherapeutics.

The MAVEN: On-line Automated Analysis and Data Processing with *In situ* Probe Sampling



Step 1: During the measurement cycle, transport buffer flow is paused and the concentration gradient drives analytes into the clean buffer solution from the medium.

Step 2: The loaded buffer is then transported to the biosensor in the measurement cell. Thereafter, the spent buffer solution is discarded into a waste container and clean buffer is sent back to the probe.

Step 3: Anodic oxidation from enzymatic conversion of glucose and lactate produces electrical signal proportional to analyte concentration. This signal is used to control feeding through the integrated PID controller or a connection to bioreactor controllers.

Figure 3: Sampling via MAVEN in-situ diffusion membrane probe
- *Requires no volume from the process*
- *Only allows small molecules to enter the stream – does not alter cell culture medium*
- *Independent of media composition (cell density, protein content etc.) and viscosity*
- *Enables fast response time and wide concentration range (dependent on measurement frequency)*

Batch Process: Bioreactor *In situ* Probes

Different diffusion probes (Fig. 4) have been developed for stainless steel and single-use bag bioreactors and fermenters. The reusable probes are autoclaved together with other bioprocess materials. Using the probe for frequent measurements reduces the risk of contamination from sample pulls and the manual work involved.

Multi-use (stainless steel)

Single-use bioreactors (via Keenpak-Adapter)

Single-use

Figure 4: Three types of MAVEN diffusion probes

Figure 5: Intensified process- Perfusion bioreactor modules and flow cells

Continuous (perfusion) processes utilize flow cells (Fig. 5) – Monitoring glucose and lactate in the spent media stream. These modules and flow cells are not in contact with the cell culture environment.

Selective Detection with Biosensor Technology

Very selective enzymatic recognition is used to detect the target analyte. Quantitation is based on the enzymatic reaction with well-established kinetics.

Enzymes are immobilized on electrode surfaces, resulting in very stable, long-term useable sensors for continuous monitoring. The biosensors are replaced after 5,000 measurements, and therefore the lifetime of a sensor is dependent on the measurement frequency. The biosensor is connected to the diffusion probe/flow cell via the tubing set and measures glucose and lactate in the loaded buffer stream hence there is no direct contact between the sensor and bioreactor medium.

Continuous Glucose and Lactate Measurements and Glucose Feeding

- Frequent measurement of main cell culture nutrients and metabolites allows for better process control

- Automated, continuous feeding of glucose enables maintenance of optimal glucose and lactate concentrations, improving growth, productivity and cell culture longevity (by reducing toxic metabolite accumulation)

- Low levels of metabolites help meet target product quality profile

- Diffusion probe and biosensor technology reduce the risk of contamination, probe clogging and provide a reliable, frequent and consistent measurements of glucose and lactate

References

1. Pereira, S., Faustrup Kildegaard H., Rørdam Andersen M.; 2018; *Impact of CHO Metabolism on Cell Growth and Protein Production: An Overview of Toxic and Inhibiting Metabolites and Nutrients*; Biotechnol. J., Vol 13; issue 3: 1700499.

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