Raman-Based Lactate Feeding Impact on Culture Characteristics and Product Quality in Mammalian Cell Cultures

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Motivation

The biopharmaceutical industry is increasingly motivated to improve process control, understanding, and repeatability using process analytical technologies (PAT). More specifically, Raman-based optical, in-line measurement methods have shown promise as a way to monitor critical performance parameters, including glucose and lactate concentrations, in real-time. Recently, this has translated to the implementation of Raman-based glucose feeding strategies to modulate productivity and product quality in Chinese hamster ovary (CHO) cell cultures.[1] The current work implements a novel Raman-based solution that does not require any chemometric models for glucose, lactate and biomass measurements. The PATsmart™ MAVERICK® System was directly, without any training in the specific bioprocess, deployed to **dynamically control lactate feeding.** We compared feeding strategies as means of modulating product quality and improving culture conditions, primarily through decreased ammonia accumulation.

Methods

Experiment Conditions

Bioreactor
Distek BIOne 1250 3 L; Biostat A plus 3 L

Cell Line
NIST-CHO mAb

Seeding Density
0.5x10^6 cells/mL; 4.5x10^6 cells/mL

Medium
Ex-Cell Advanced CHO Fed-batch Medium (SAFC)

Feed
Ex-Cell Advanced CHO Feed 1 (SAFC) w/o glucose fed at 5% EOD

Glucose Feeding Strategy
Bolus feed with threshold <4 g/L, supplement to 6 g/L

Lactate Feeding Strategy
1. 1.5 g/L lactate set point after consumption begins
2. No lactate feed

Analytics
- **In-line:** MAVERICK Raman-based real-time glucose, lactate, and biomass
- **At-line:** Vi-CELL (Beckman Coulter), BioProfile FLEX2 (Nova Biomedical), and REBEL for amino acids
- **Off-line protein quality attributes:** ZipChip coupled to Orbitrap Exploris 240 Mass Spectrometer (Thermo Scientific)

Experiment Goals

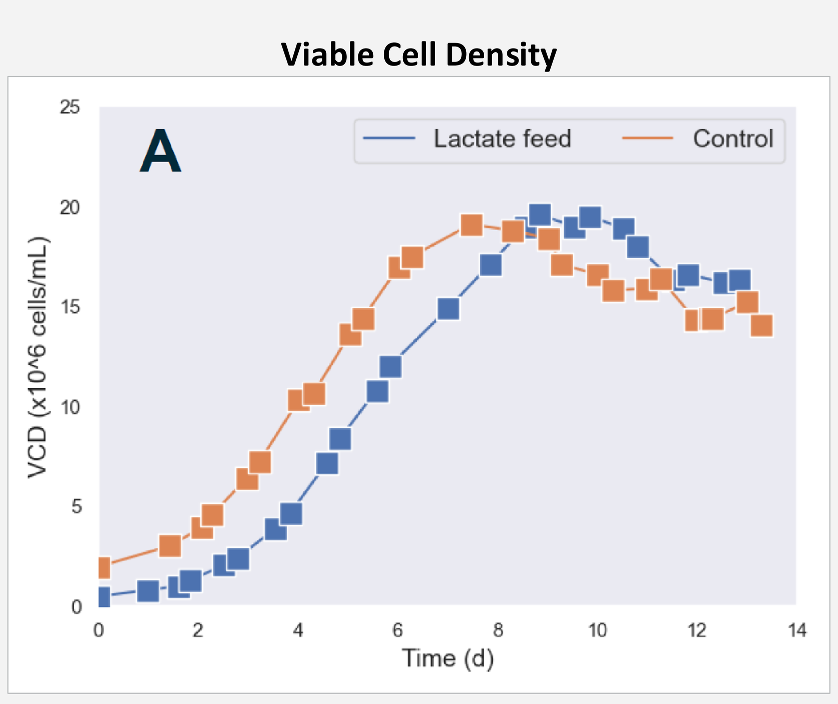
Goal
- Raman-based measurements used for dynamic lactate feeding for decreased ammonia accumulation in CHO cell culture
- Comparing 1.5 g/L lactate set point to no lactate feed
- Both conditions had glucose bolus feed

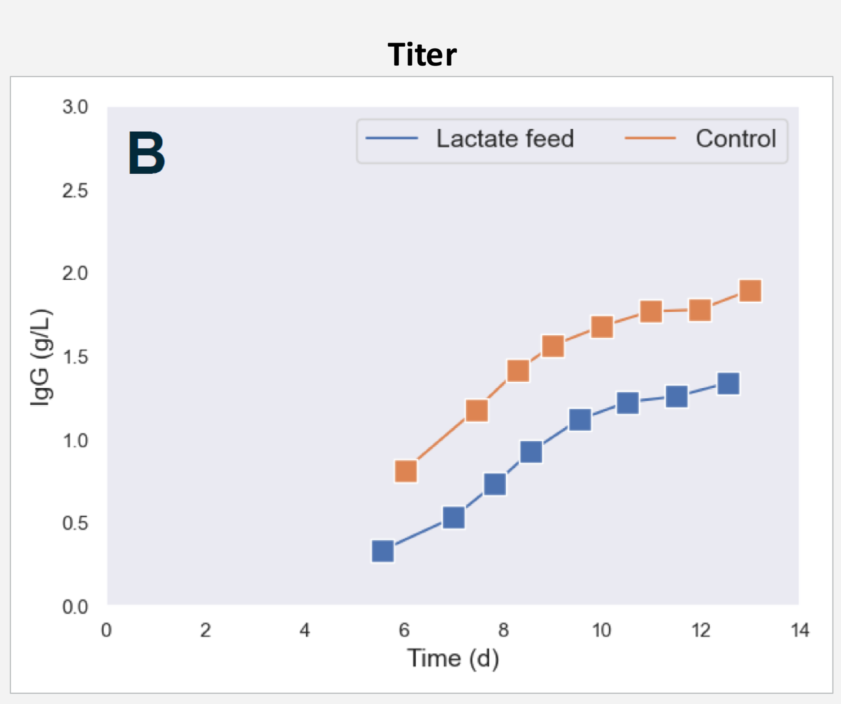
PATsmart™ MAVERICK®
In-line glucose monitoring and lactate control

PATsmart™ REBEL® XT
At-line amino acids

PATsmart™ ZipChip®
CQAs

Growth and Productivity





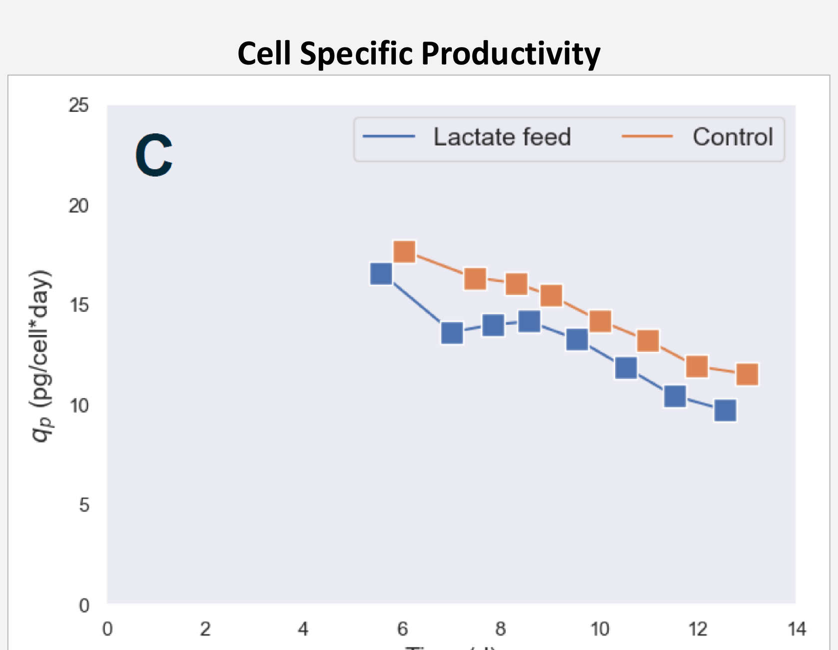
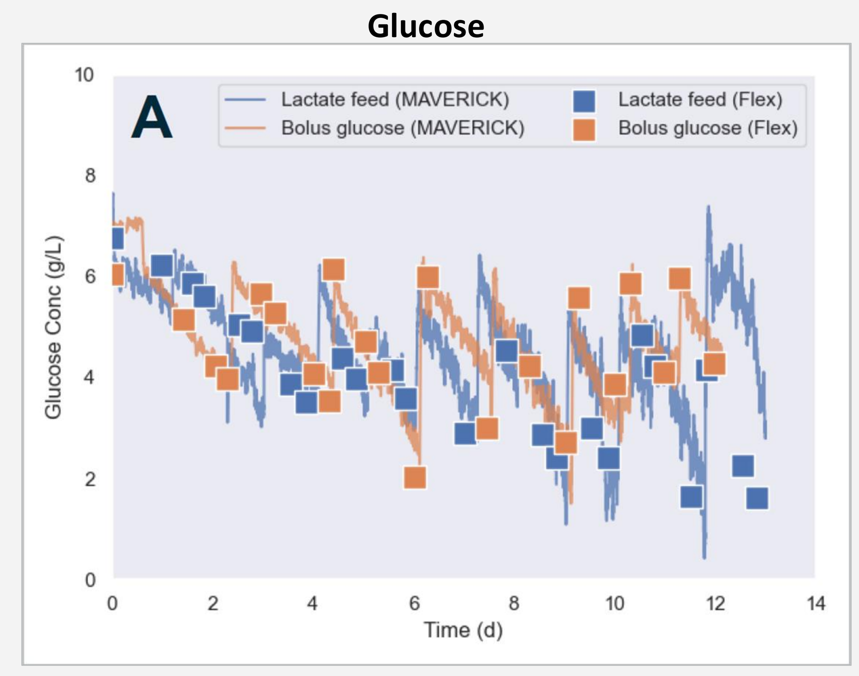
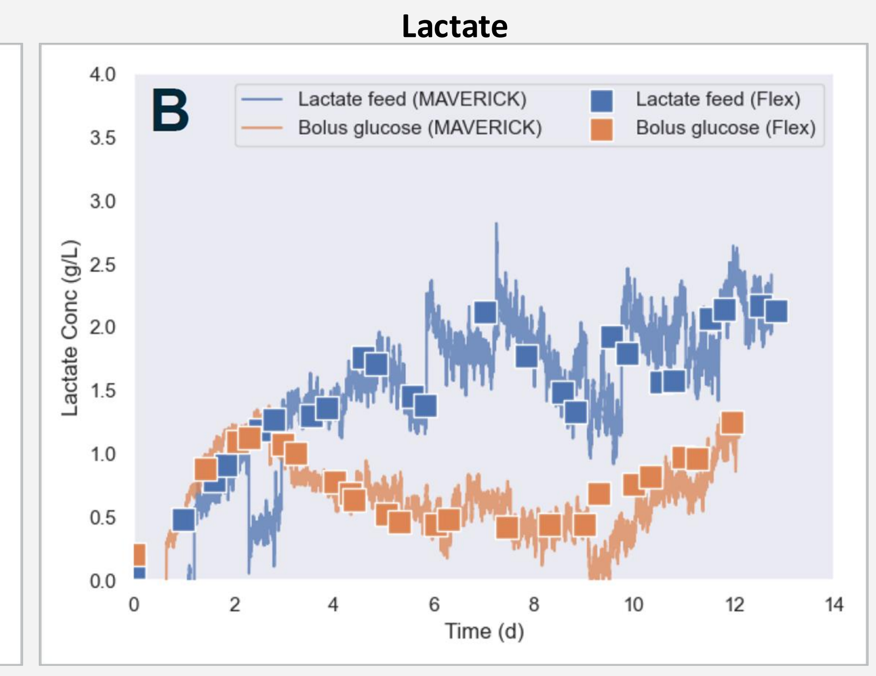


Figure 1. Lactate feeding impact on growth and productivity: A) Viable cell density (VCD), B) Titer, and C) Cell specific productivity (q_p) for control (orange) and lactate fed (blue) conditions. The lactate feed resulted in **comparable max VCD, but lower final titer and q_p** likely due to lower seeding density of the culture.

Metabolites





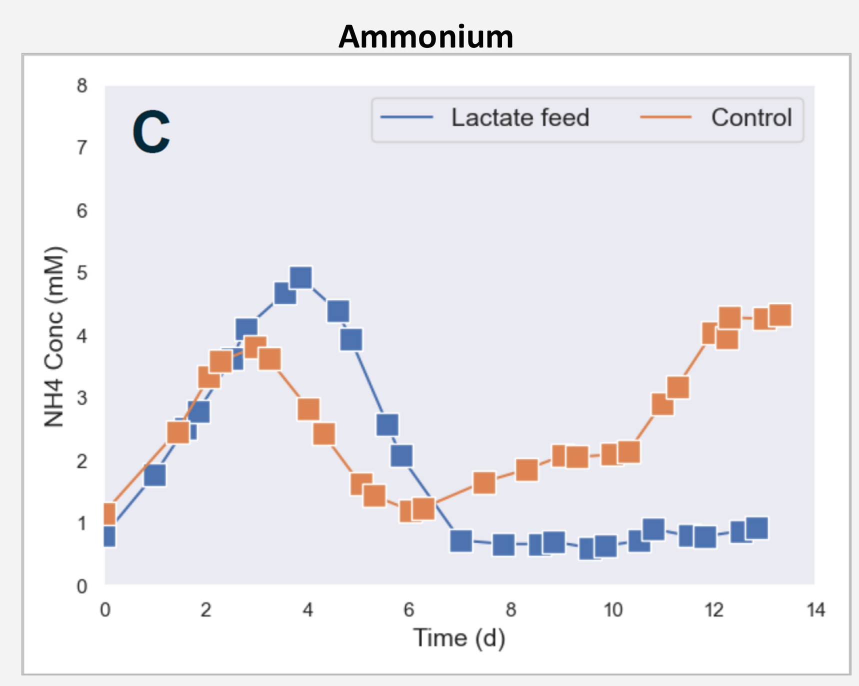
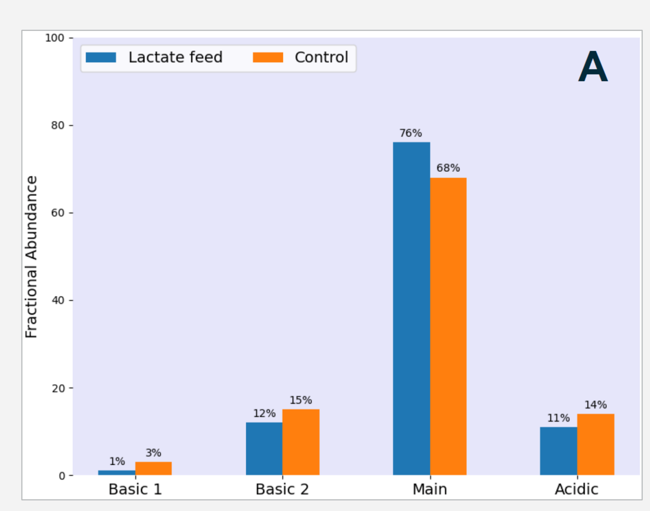


Figure 2. Lactate feeding impact on metabolites: A) Glucose, B) Lactate, and C) Ammonium concentrations for control (orange) and lactate fed (blue) conditions. The **lactate feed successfully reduced the ammonium** accumulation at the end of the culture.

Product Quality



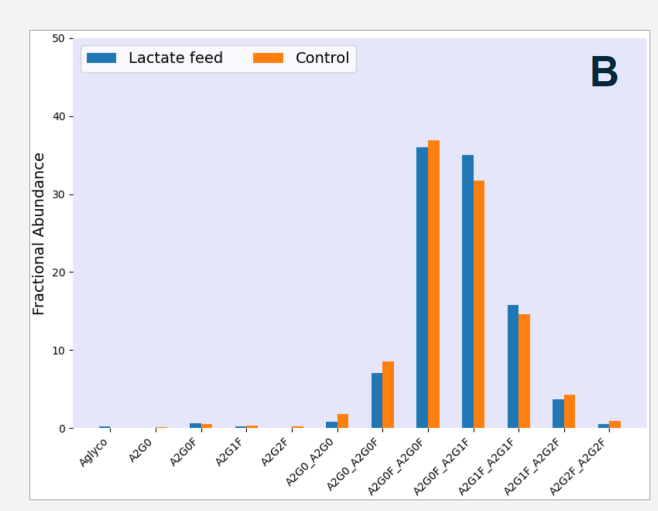
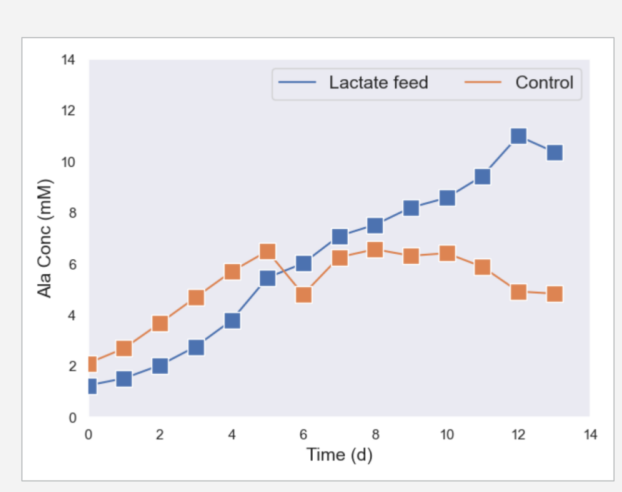


Figure 3. Lactate feeding impact on product quality: A) Charge variants and B) glycosylation for control (orange) and lactate fed (blue) conditions. The **lactate feed resulted in a higher percentage of mAbs in the main peak in the charge variant profile, as well as a higher abundance of more mature (G1 and G2) glycoforms.**

Alanine Profiles



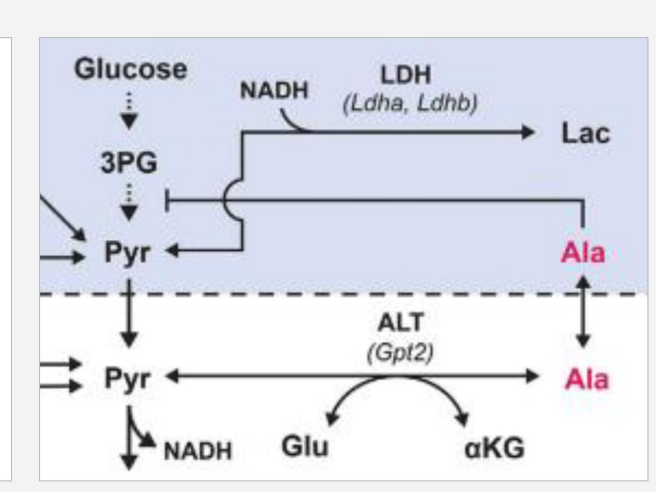


Figure 4. Lactate feeding impact on alanine levels: Sufficient lactate supply in the lactate fed cultures blocks a switch to alanine consumption. Instead of metabolizing alanine, lactate is used as a pyruvate source, leading to reduced levels of free ammonium from alanine metabolism.

Conclusions

- Easy and instant implementation of lactate control with MAVERICK
- Improved cell culture performance: ammonium profile
- Improved protein quality attributes: more mature, heterogeneous quality attribute profiles

References

1. Domján J, Fricska A, Madarász L, et al. Raman-based dynamic feeding strategies using real-time glucose concentration monitoring system during adalimumab producing CHO cell cultivation. *Biotechnology Progress*. 2020.

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