Enabling Smarter Fed-Batch Bioreactors Operations with In-Situ Near-IR Process Analytics

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Abstract: Improving visibility into metabolite dynamics during fed-batch operation is critical for advancing process understanding, robustness, and control in biotherapeutic manufacturing. To support this goal, Culture Biosciences and Nirrin Technologies evaluated an *in-situ* Near-Infrared (Near-IR) process analytical technology (PAT) probe for monitoring key process variables during a CHO fed-batch process using NISTCHO cells in the Stratyx™ 250 mL cloud-connected bioreactors. Near-IR measurements of glucose and lactate were compared against established offline analyzers, including Flex2 and CEDEX, and showed strong agreement in both magnitude and temporal trends. IgG titer trends measured by Near-IR were consistent with offline analytics, while viable cell volume measurements aligned with ViCell Blu results. Data analysis was performed using a library-based spectral approach that leverages known component signatures, reducing reliance on large empirical calibration models commonly required for Raman-based PLS workflows. Once data was analyzed it was automatically imported into a cloud-based software that allowed users to access and visualize it from anywhere. This framework enables robust, interpretable quantitation while remaining adaptable to evolving process conditions. Together, these results demonstrate the utility of *in-situ* Near-IR spectroscopy as a practical PAT tool for monitoring critical metabolites and cell culture attributes directly within the bioreactor, supporting improved process insight and the potential for intelligent fed-batch control strategies.

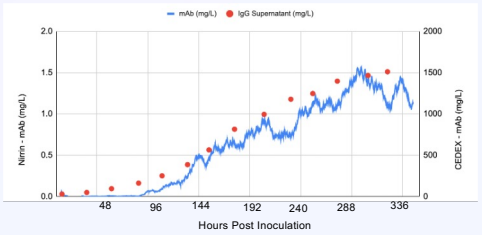
Materials and Methods:

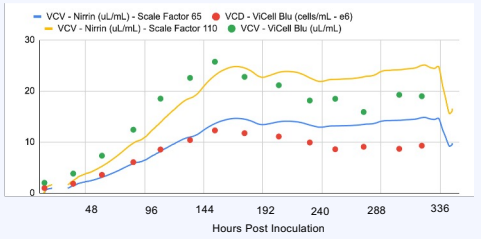
- Cell Line: NISTCHO-CB-BRD-1A
- Batch Media: EX-CELL Advanced CHO Fed-batch Medium + 6mM Glutamine
- Glucose concentration: 450g/L
- Nutrient Feed: EX-CELL Advanced CHO Feed 1 (w/o glucose)

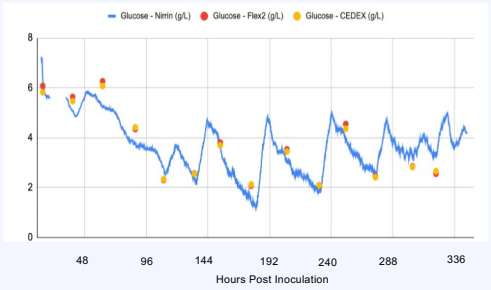
Bioreactor Process Parameters

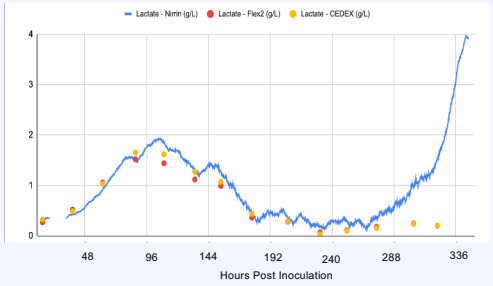
Target Inoculation Viable Cell Density	1.0 x 10^6
Post-Inoculation Vessel Volume	200
Length of Fed-batch Culture	14
Glucose Feed; Threshold/Target (g/L)	Day 3 - Day 13; 3/6
Nutrient Feed (odd days); % cWV	Day 3 - Day 13; 2.5%
DO Setpoint (%)	40
Agitation Speed (rpm) - Downflow	300+
pH Setpoint	6.90 +/- 0.10
pH Control	1N NaOH or CO2
Temperature Setpoint (C)	36.5

Results: Near-IR probes in bioreactors were compared to various off-line in process analytics including chemistry on Flex2, Viable cell density on ViCell Blu, as well as glucose and lactate in CEDEX. Probe data was in line with expectations for all the offline analytics. With offsets to evaluate viable cell volume and viable cell density which offers developers alternate ways of looking at cell health and growth.









Conclusions:

- in-situ Near-IR spectroscopy as a practical PAT tool for monitoring critical metabolites and cell culture attributes directly within the bioreactor
- Modern bioreactors that are built to be integrated with PAT tools can drive better process development, faster.

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