Impact of Dynamic Online Fed-Batch Strategies on Metabolism, Productivity and N-Glycosylation Quality in CHO Cell Cultures

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Abstract

In an effort to improve yields in order to meet the growing demands of therapeutics, the impact of process controls on glycosylation patterns can be employed as a means of ensuring increased efficacy and consistency. By utilizing an online fed-batch model based on maintaining levels of glutamine/glucose; impact on cellular metabolism, productivity, and N-glycosylation quality of the model recombinant protein, interferon gamma (IFN-*y*), can be quantified. Glutamine concentrations of 0.3mM provided a 10-fold increase in yield, and maintained an unequivocal macro- and microheterogeneity of IFN-*y*. It was also observed that low concentrations of glutamine and glucose (<0.1mM and <0.7mM respectively) led to decreased sialylation and increased presence of minor glycan species. In addition to nutrient limitation, N-glycosylation can be adversely affected by decreased cell viability and presence on inhibitory lactate and ammonia. Therefore it is imperative to measure both the culture viability as well as nutrient set points in order to optimize N-glycosylation quality.

Cell Culture Process

Cell Line & Medium

- Recombinant CHO cells producing interferon gamma (IFN-*y*)
- Batch Media: HyClone CHO MPS

Bioreactor Operation

- Bioreactor: glass with anchor/marine impellers
- Inoculum density: 0.25 E6 cells/ml
- Working volume: 4.0L
- Temperature: 37°C
- pH: 7.15
- Batch phase: Day 7

Feed Control System

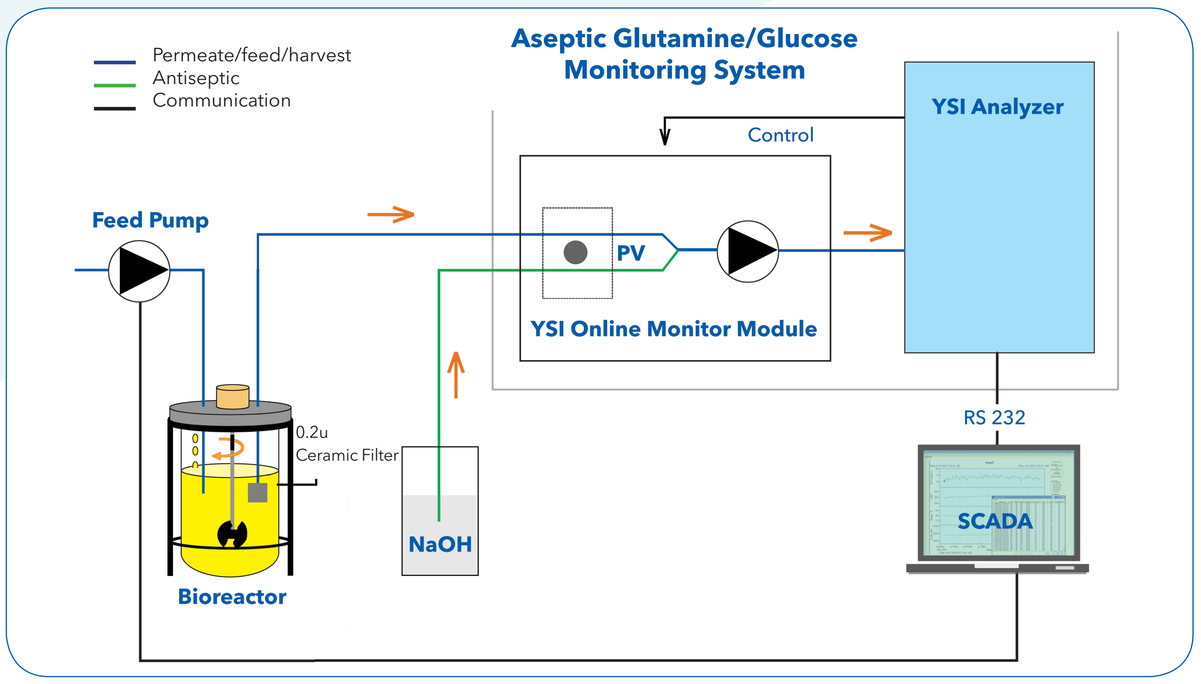
- SCADA controlled feed pump rates to maintain glucose and glutamine concentration at prescribed setpoints
- Basal feed media to provide 5:1 concentration of glucose:glutamine
- Several independent concentrations measured
  - 0.1mM Glutamine
  - 0.3mM Glutamine
  - 0.5mM Glutamine

On-line Monitoring System

- Glutamine and glucose analysis of cross-flow filtered permeate
- YSI biochemistry analyzer configured with glucose oxidase, lactate oxidase, glutaminase/glutamate oxidase membrane electrodes
- On-line monitoring module configured with peristaltic pump and dual pinch valve
- Sample/Analytical Cycle
  - Analyzer autocalibration
  - Activate pinch valves and permeate (sample) for 6 minutes (purge)
- Sample delivered to analyzer sample cup
- Sample analyzed for glucose and glutamine
- Analyzer data communicated to SCADA via RS-232
- Switch pinch valve and pump Antiseptic through sample line and sample cup
- Antiseptic remains in line and sample cup until next sample cycle.

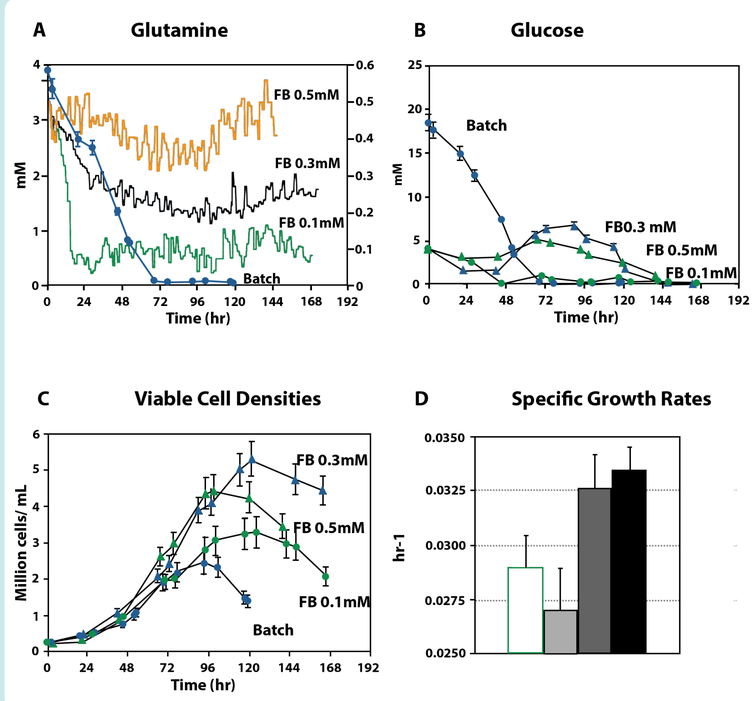
YSI 2900M
On-line Monitoring & Control System

On-line Glucose Monitoring and Control: Closed-loop System



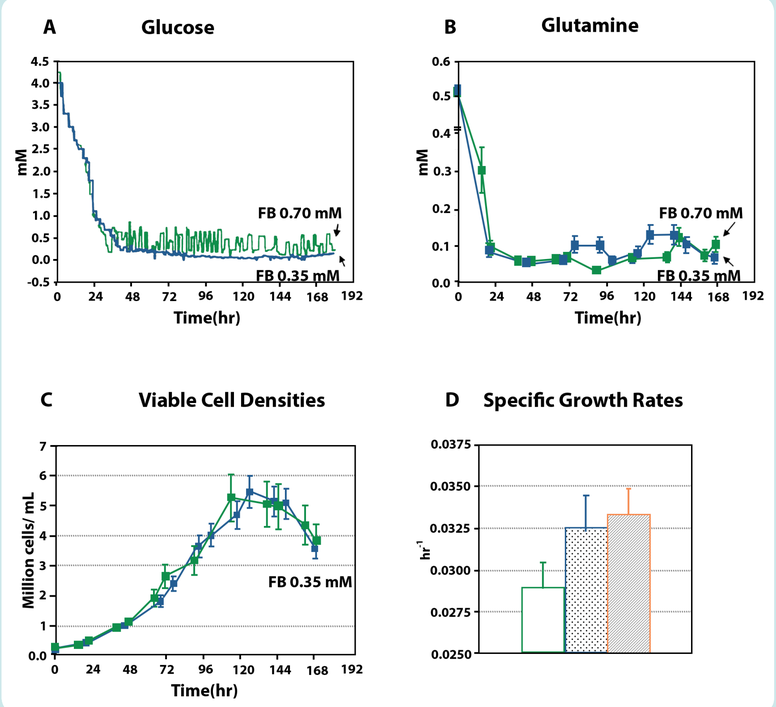
Results

Growth Kinetics of Glutamine Setpoint Fed-Batch Cultures



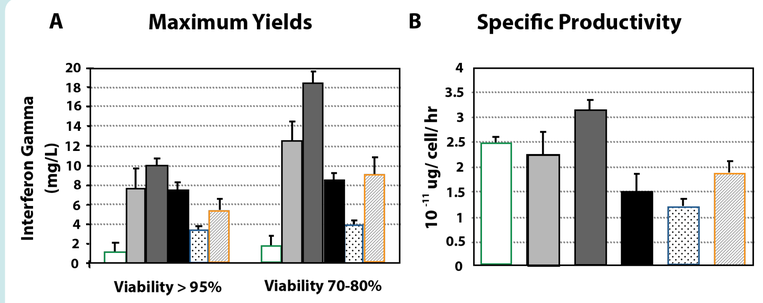
Concentrations of (**A**) on-line residual glutamine and (**B**) off-line residual glucose with (**C**) viable cell densities of fed-batch cultures controlled at 0.1 mM (●), 0.3 mM (▲), and 0.5 mM (▲) glutamine, and control batch (●) culture. **D**: Average specific growth rates, μ, for batch ■ , glutamine fed-batches at 0.1 mM ■, 0.3 mM ■, and 0.5 mM ■(data points represent the averages of two runs). Lactate and ammonium data not shown.

Growth Kinetics of Glucose Setpoint Fed-Batch Cultures Coupled with Glutamine Profile Feeding



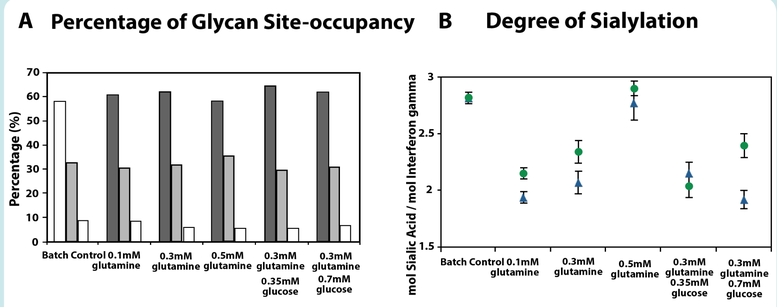
Concentrations of (**A**) On-line residual glucose and (**B**) Off-line residual glutamine with (**C**) Viable cell densities of fed-batch setpoint cultures controlled at 0.35mM (■) and 0.70mM (■) glucose coupled with glutamine profile feeding. (**D**) Average specific growth rates, μ, for batch ■ and fed-batch cultures controlled at 0.35mM ▨ and 0.70mM ▨ glucose coupled with glutamine profile feeding. (Data points represent the averages of two runs). Lactate and ammonium data not shown.

Interferon -*y* Yields and Productivity



Recombinant human IFN-*y* production in CHO cells during batch and fed-batch cultures. **A**: Maximum IFN-g yields during high and low viability for batch culture ■ and glutamine setpoint fed-batch cultures controlled at 0.1 mM ■, 0.3 mM ■, and 0.5 mM ■ glutamine and for 0.3 mM/0.35 mM ▨ and 0.3 mM/0.70 mM ▨ glutamine/glucose fed-batch cultures (data points represent the averages of two runs).**B**: Average specific IFN-*y* productivity rates.

Glycan Site Occupancy and Degree of Sialylation



A: Proportion of 2-N ■ , 1-N■, and 0-N□glycan siteoccupied IFN-*y* in batch and fed-batch cultures. **B**: Sialic acid content of maximum IFN-*y* harvested during high viability, >95% (●) and low viability, 70–80% (▲) in batch and fed-batch cultures (data points represent the averages of two runs).

Conclusions

- **Dynamic glutamine or glucose/glutamine controls are effective strategies for enhancing cellular metabolism by decreasing metabolite waste production.**
- **Feed Control Strategies increase cell viability and productivity**
- **N-glycosylation and sialylation can be effectively enhanced through process control.**

Acknowledgements
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