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Abstract

Among the variables that are appropriate for direct feedback control of the perfusion rate in mammalian cell cultures, high priority should be given to the glucose concentration. Here we describe the application of a closed-loop control scheme for the long-term cultivation of CHO cells in a high cell density (35 - 40 million cells/ml) perfusion process. The monitoring and control system worked successfully for 2.5 months without any signs of performance degradation. In targeting industrial applications, issues such as reliability, sterility and accuracy are given high priority. The implementation of the glucose monitoring system, which is the main part of the control complex, is addressed. The performance of the perfusion culture was evaluated at four different glucose set points, providing essential information about process optimization. The on-line glucose concentration was used by an embedded expert system which drove the process through the batch and the perfusion phases, achieving total SCADA control of the feed rate. In summary, the proposed glucose monitoring and control technique proved to be a reliable tool which can be applied with confidence at an industrial scale for either microbial or mammalian cell cultures.

Rationale

Evaluation of a closed-loop glucose-stat system for perfusion rate control in long-term cultivation of a high density CHO cell perfusion process.

Cell Culture Process

Cell Line & Medium

- Recombinant CHO cells producing truncated intercellular adhesion molecules (ICAMs)
- Proprietary medium buffered with NaHCO₃

Bioreactor Operation

- Bioreactor: stainless steel w/ anchor/marine impellers
- Inoculum density: 0.5 E6 cells/ml
- Working volume: 12L
- Temperature: 37°C
- pH: 6.9 - 7.1
- Batch phase: Day 0 - 2
- Perfusion/fed-batch phase: Day 2 - Harvest

Perfusion System

- SCADA-controlled feed and harvest pump rates to maintain glucose concentration at the prescribed setpoints
- Manipulation of feed and harvest rates maintain desired glucose concentration using constant feed control algorithm
- On-line glucose concentration used by SCADA to drive process through both batch and perfusion phases
- Glucose concentration setpoint/Average dilution rate:
 - 2.0 g/L/~ 3.6 volumes/day
 - 2.5 g/L/~ 5.0 volumes/day
 - 3.0 g/L/~ 5.7 volumes/day
 - 3.5 g/L/~ 5.7 volumes/day

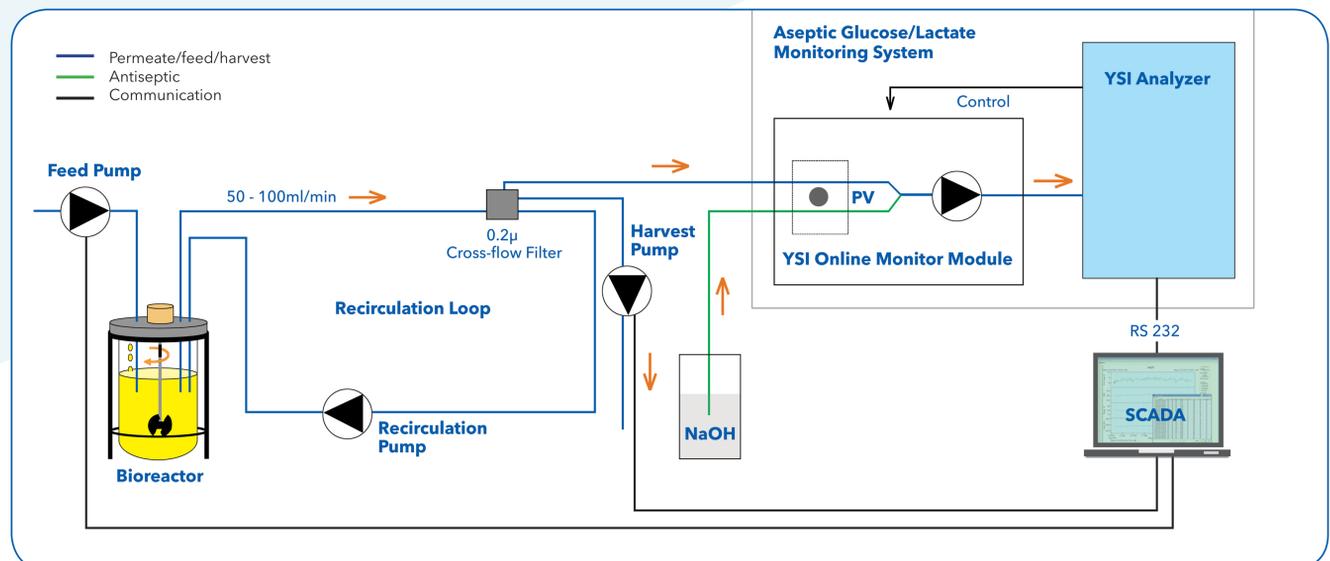
On-line Glucose Monitoring system

- Simultaneous glucose and lactate analysis of cross-flow filtered permeate (0.22 µm).
- YSI Biochemistry Analyzer configured with immobilized glucose oxidase and lactate oxidase membrane electrodes.
- On-line monitoring module configured with peristaltic pump and dual pinch valve
- Sample/Analytical Cycle
 - Analyzer autocalibration
 - Activate pinch valve and pump permeate (sample) for 6 minutes (purge)
 - Sample delivered to analyzer sample cup
 - Sample analyzed for glucose and lactate
 - Analyzer data communicated to SCADA via RS-232
 - Switch pinch valve and pump antiseptic (NaOH) through sample line and analyzer sample cup
 - Antiseptic remains in line and sample cup until next sample cycle



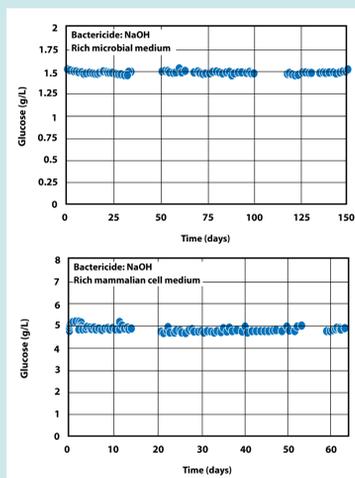
YSI 2900M
On-line Monitoring & Control System

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



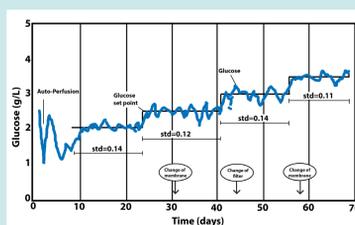
- Real-time data used for direct feedback control of the perfusion rate
- No signs of performance degradation during cultivation period

Sterility Tests of the Glucose Monitoring System



- Glucose concentration of cell-free medium in sterile vessels was monitored by YSI analyzer online monitoring system
- Regular sample analysis confirmed absolute sterility was maintained throughout the experiment duration.

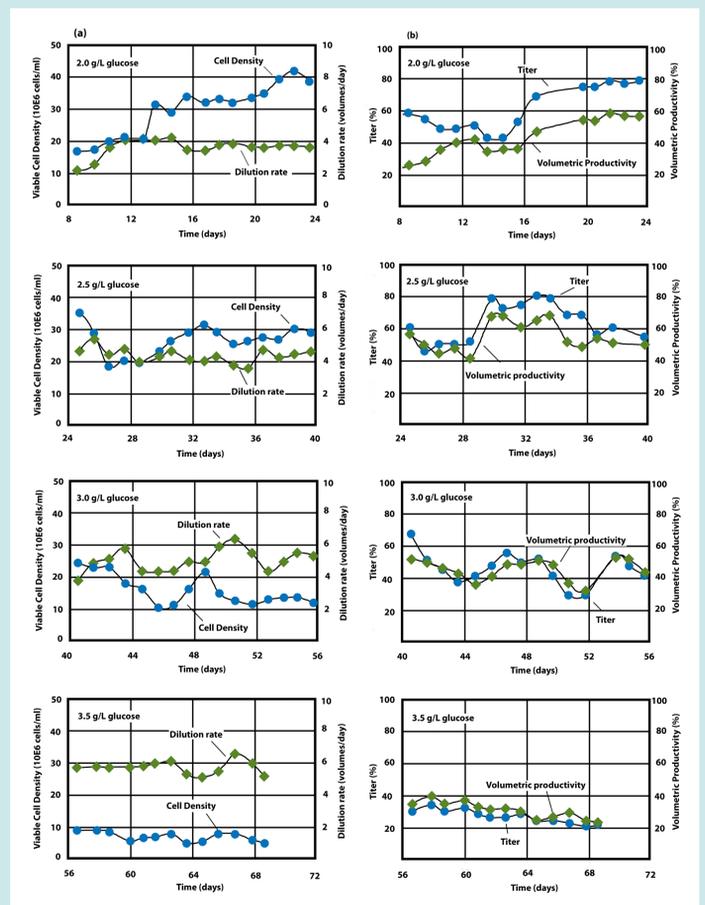
Time Profile of Glucose Setpoints



- Perfusion culture performance evaluated at four different glucose set points - 2.0, 2.5, 3.0, and 3.5 g/L
- Each setpoint maintained approximately 2 weeks
- Standard deviation values indicate glucose was accurately controlled around each setpoint.
- Oscillations in days 25 - 56 caused by decreased volumetric glucose uptake rate due to dilution effect

Results

Cell Density, Dilution Rate, Titer and Volumetric Productivity



- Time profiles of (a) cell concentration & perfusion dilution rate, and (b) relative product titer and relative volumetric production rate
- Glucose concentration increases correlate to increased dilution rates
- Reduction in cell density, product titer and volumetric productivity observed with increased dilution rates
- Decreasing trend of product titer and volumetric productivity caused by decreased cell density due to increased dilution rate (Days 41 - 70)

Conclusions

- Long-term reliability and control of perfusion CHO cell culture by an aseptic glucose monitoring and control system
- Method proven for 1) long-term stability, 2) accurate analytics, 3) absolute sterility, 4) completely automatic operation, 5) simple process interface, 6) small footprint and 7) low maintenance
- On-line glucose/lactate concentration data useful for process optimization, control and cell physiology studies
- Monitoring technology mature and reliable for small and large-scale mammalian and microbial processes

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