Enhancing Automated Sampling, Process Monitoring, and Nutrient Feedback Control for a System of 3-L Bioreactors

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Abstract

Background and Novelty

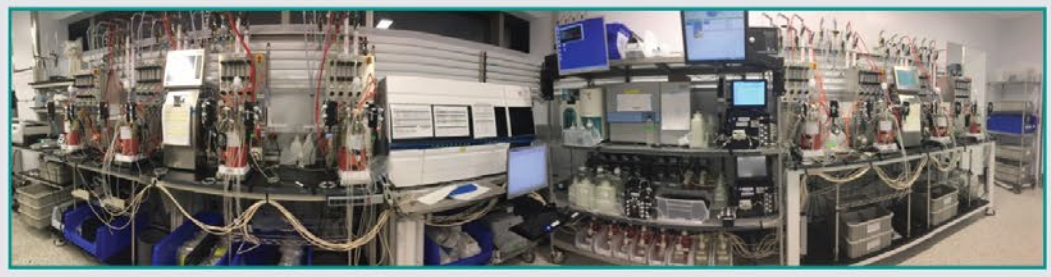
Key to effective upstream development is the presence of resource-efficient systems for examining complex process options. In this presentation, we report on the progress of a completely automated sampling and feed control system capable of simultaneously evaluating different metabolic-based feed strategies for bench-scale cell culture processes. Though there are automated feed systems available for microscale vessels, to date there is not a complete 3-L scale sample and feed automation solution as described here, with capability to transfer samples from multiple bioreactors (single-use or glass) to a suite of independent analyzers with concurrent feedback control of nutrient feed pumps. There are multiple drivers for developing automated systems for benchtop bioreactors, ranging from increased operational efficiency to enhancing the toolbox for process development. Deeper process understanding, obtained through examining metabolic-tailored feeding strategies, is essential to the development of state-of-the-art upstream processes with emphasis on product quality attributes.

Experimental Approach

The automated online sampling and feed system is capable of feedback control for a variety of complex feed strategies that incorporate data inputs from integrated third-party analyzers. Feed control inputs can be cell density and/or up to four measured residual metabolites. The completely automated system has supported experiments from two to eight 3-L bioreactors, with sampling frequencies ranging from 4 to 24 hours per tank, depending upon experimental needs.

Results and Discussion

In a recent typical experiment, eight tanks were sampled three times per day with feedback control for multiple separate nutrient feed solutions per tank, based upon independent metabolite triggers. Over the course of the run, each vessel was triggered feeds 35 to 40 times with feed volumes ranging from 2 to 40 mL for over ~300 automated feeds across the eight vessels. Refined data extraction templates streamlined data imports into the electronic notebook system, and PI integration can enable remote monitoring. The enhanced system capabilities are being increasingly leveraged to support ongoing process development efforts.



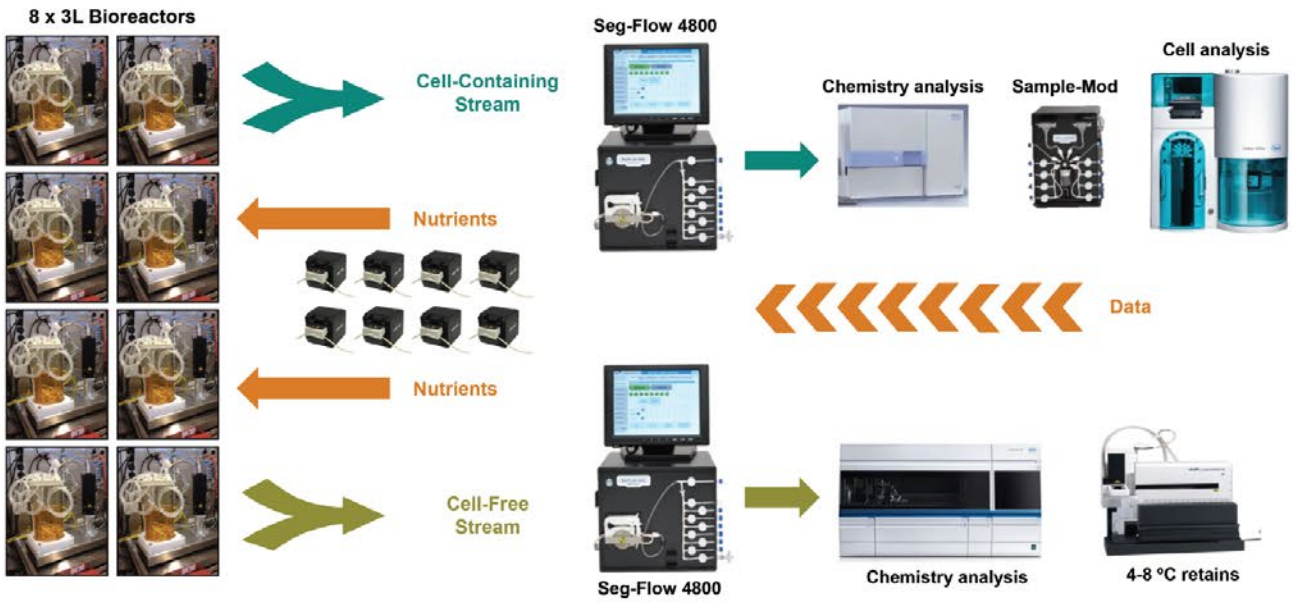
Automated Sampling
- Sample volumes 1 to 15 mL
- User-defined frequency and volume
- Completely disposable fluid path
- High sterile fidelity

Dual Sample Streams
- Cell-containing and cell-free sample streams pulled from each reactor
- 16 sample inputs for dual Seg-Flows

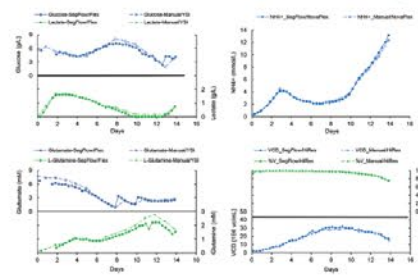
Integrated Analyzers
- Integrated with
  - Cedex HiRes
  - Cedex Bio HT
  - Nova Flex
  - Fraction Collector
- Seg-Flow logs data for each integrated analyzer

Feed Control
- Calculates feed requirements and activates pumps based upon integrated analyzer data and user inputs
- Four automated feeds per reactor

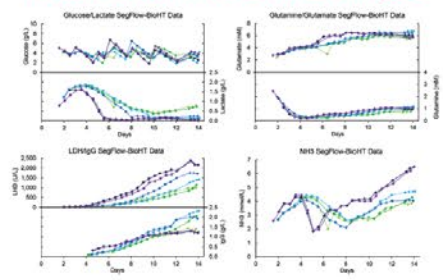
Flexible Data, Connectivity, and Integration Options
- Customized data export to streamline upload to electronic notebook
- Real-time data imported to PI historian for remote access 24/7



Successful Online Process Monitoring



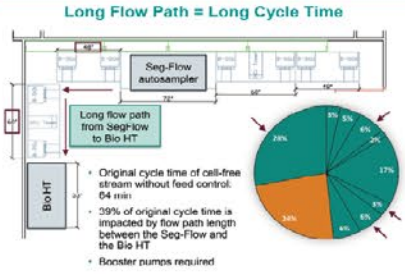
Achieved Automation of Bio HT Analysis



Seg-Flow to Bio HT: six tanks sampled every 12 hours for 14 days

IMPACT OF SYSTEM LAYOUT ON CYCLE TIME AND SAMPLE DELIVERY

Long Flow Path = Long Cycle Time



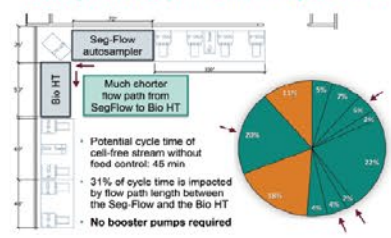
- Original cycle time of cell-free stream without feed control: 64 min
- 39% of original cycle time is impacted by flow path length between the Seg-Flow and the Bio HT
- Booster pumps required

Bio HT Sensitive to Bubbles in Flow Cell

- "No fluid in sample cup" error – some tests failed to run
- Order of errors indicates bubbles rather than empty flow cell
- Mitigated with several changes
  - New flow cell
    - Steeper angle and higher polish to reduce bubble retention
  - New sample delivery paradigm – required shorter flow path

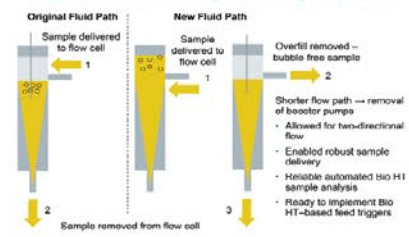
Desired resolution to enable Bio HT–triggered feed control

New System Footprint Reduced Cycle Time



- Potential cycle time of cell-free stream without feed control: 45 min
- 31% of cycle time is impacted by flow path length between the Seg-Flow and the Bio HT
- **No booster pumps required**

Achieved Robust Sample Delivery to Bio HT

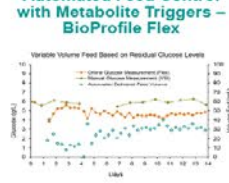


FEED CONTROL

Current Automated Feed Schemes
- Fixed volume – time based
- Residual metabolite

  Triggers a feed when below target value for designated metabolite
  - Fixed-volume bolus
  - Calculated-volume bolus

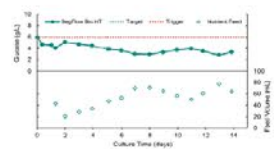
Automated Feed Control with Metabolite Triggers – BioProfile Flex



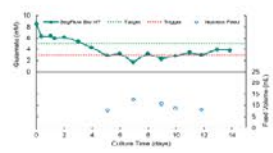
Demonstrated Automated Feed Stats
- Eight vessels over 14 days
- 35 to 40 feeds per vessel
- Two metabolite triggers per vessel
- Feed volumes from 2 to 40 mL per bolus
- ~300 automated feeds fed 5+ liters

Automated Feed Control with Metabolite Triggers – Cedex Bio HT

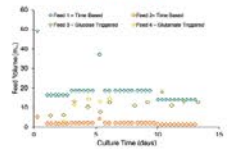
Glucose



Glutamate



Success of Four Automated Feeds Per Reactor

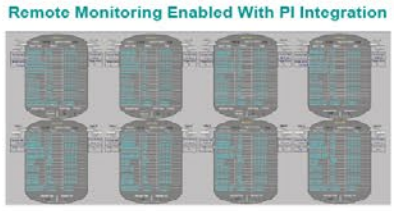


Feed Control - Next Steps:
- Cell density based triggers
- Continuous feed algorithm
- Balance integration for real-time working volume

- Two multicomponent feeds added at fixed volumes (time based)
- Two single-component solutions supplemented based on metabolite triggers

REMOTE MONITORING ENABLED BY INTEGRATION OF SEG-FLOW WITH PI HISTORIAN

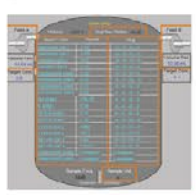
Remote Monitoring Enabled With PI Integration



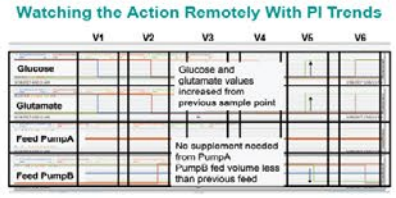
- Display files built in ProcessBook for viewing tag values
- Display files can be viewed in ProcessBook or Coresight

Remote Monitoring: A Closer Look

- Cell free Seg Flow display example
- Last logged value for any PI tag
- Seg-Flow status
- Sample volume
- Bio HT test results
- Bio HT result flags
- Volumes fed



Watching the Action Remotely With PI Trends



- Configured trends to display step change between points
- Can see remotely, at a quick glance, how metabolite values and feed volumes are changing each sample cycle

SUMMARY

Innovative effort led to sophisticated bench-scale automation
- Eight 3-L bioreactors
- Cell-free/cell-containing samples
  - Typical purge/sample volumes: 2 to 7 mL
- Three instrument analyzers
  - Roche Cedex HiRes
  - Roche Cedex Bio HT
  - Nova BioProfile Flex
- Four automated feeds per reactor
  - Time-based feed option
  - Two residual metabolite-triggered feed options
- Refrigerated sterile retains
- Custom-collated data format for easy export/import
- Remote monitoring enabled by PI integration

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