Improving Cell Culture Productivity with a SEG-FLOW® Automated On-line Sampling and Feed System

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Abstract

Process analytical technology tools, such as on-line nutrient and metabolite analysis, can significantly increase process understanding while improving process control and performance. In this study, a SEG-FLOW® automated bioreactor sampling and feed system (SEG-FLOW) was evaluated for its impact on Chinese Hamster Ovary (CHO) cell culture process performance. Cell-free samples were obtained from a bench-scale bioreactor through a FISP® sampling probe and subsequently delivered to a YSI 2700 biochemistry analyzer for glucose analysis. Glucose concentration data were communicated from the analyzer to the SEG-FLOW for feed algorithm calculation and feed pump control. Media glucose levels were maintained at the desired setpoint using a semi-continuous feed scheme programmed by the SEG-FLOW software. A second batch, using a daily manual sampling and bolus feed protocol, was performed as a control. The SEG-FLOW sampled culture, as compared to the control, provided optimal glucose feeding for the CHO cells. This allowed a greater carbon conversion efficiency that reduced glucose uptake requirements, increased peak and total viable cell concentrations and increased protein yield. Reduced lactate production was also achieved, which reduced base addition requirements and lowered media osmolality. The SEG-FLOW provided an innovative approach for improving cell culture productivity through better process monitoring and control, while increasing process knowledge and understanding through real-time sample analysis.
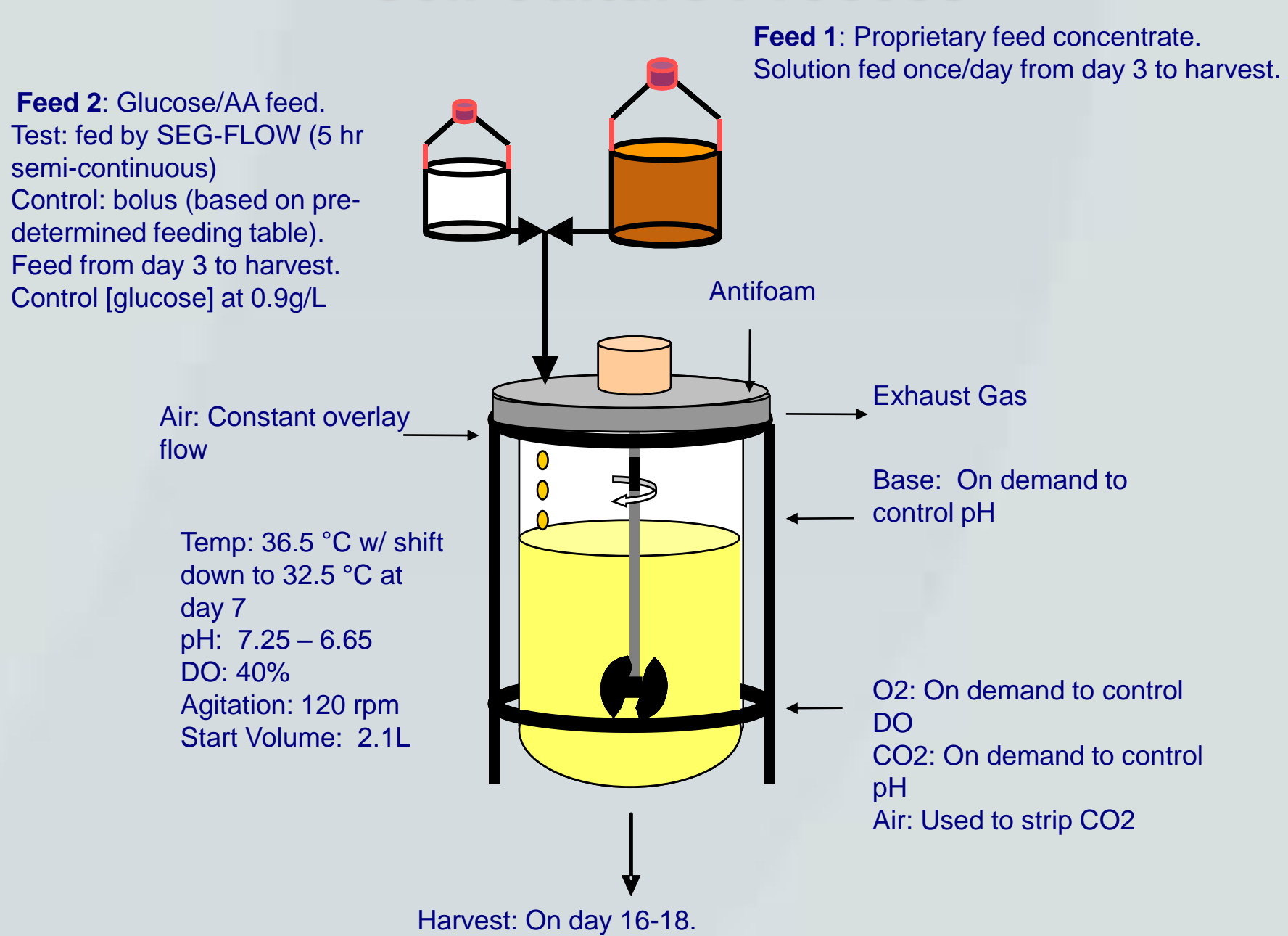
Experimental Plan

Rationale:
• Evaluate the impact of automated on-line sampling and feed control on a CHO cell culture process used for recombinant protein manufacturing.
• Increase process understanding, especially with regard to the role of glucose metabolism on recombinant protein production in a CHO cell culture.

Evaluation:
• Test Culture: Automated on-line monitoring of glucose and feedback control, semi-continuous glucose/amino acid feed would be performed by the Seg-Flow system.
• Control Culture: Manual off-line glucose analysis and a daily bolus of glucose/amino acid feed would be used.

Cell Culture Process

Feed 1: Proprietary feed concentrate. Solution fed once/day from day 3 to harvest.

Feed 2: Glucose/AA feed. Test: fed by SEG-FLOW (5 hr semi-continuous) Control: bolus (based on pre-determined feeding table). Feed from day 3 to harvest. Control [glucose] at 0.9g/L

Antifoam

Air: Constant overlay flow
Exhaust Gas
Base: On demand to control pH

Temp: 36.5 °C w/ shift down to 32.5 °C at day 7
pH: 7.25 – 6.65
DO: 40%
Agitation: 120 rpm
Start Volume: 2.1L

O2: On demand to control DO
CO2: On demand to control pH
Air: Used to strip CO2
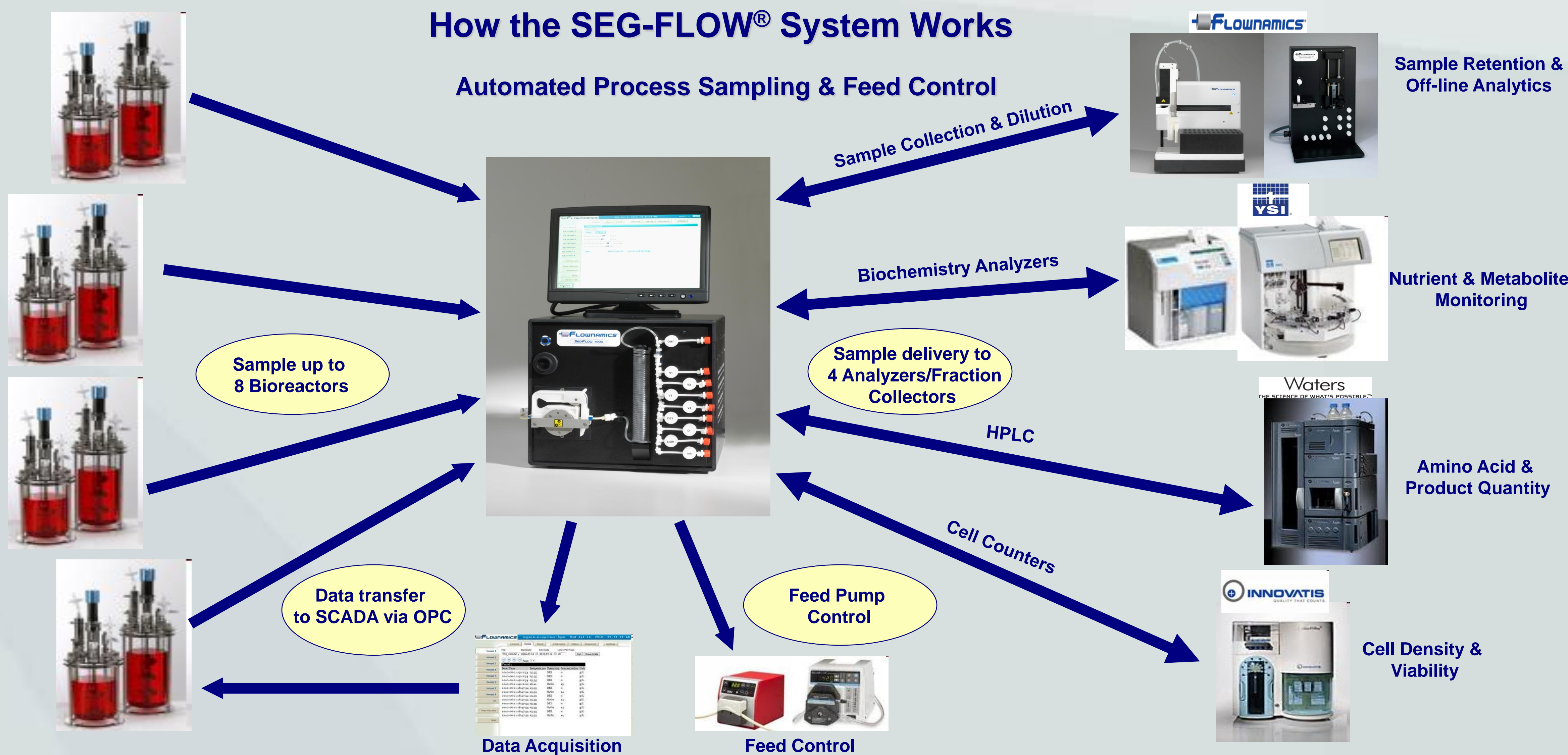
Harvest: On day 16-18.

Process Analytics

On-Line

• Seg-Flow - automated online sampling & setpoint feed control
• YSI 2700 Biochemistry Analyzer – glucose monitoring

Off-line

• Agilent 1100 Protein A HPLC – product titer
• Agilent 1200 RP-HPLC – product quality (clipping profile)
• Nova Bioanalyzer – metabolite monitoring
• Blood Gas Analyzer – pO2 & pCO2 monitoring
• Cedex Cell Counter – cell density & viability

How the SEG-FLOW® System Works

Automated Process Sampling & Feed Control

Sample Collection & Dilution
Sample up to 8 Bioreactors
Sample Retention & Off-line Analytics

Biochemistry Analyzers
Nutrient & Metabolite Monitoring

Sample delivery to 4 Analyzers/Fraction Collectors

HPLC
Amino Acid & Product Quantity

Cell Counters

Data transfer to SCADA via OPC
Feed Pump Control

Cell Density & Viability

Data Acquisition
Feed Control
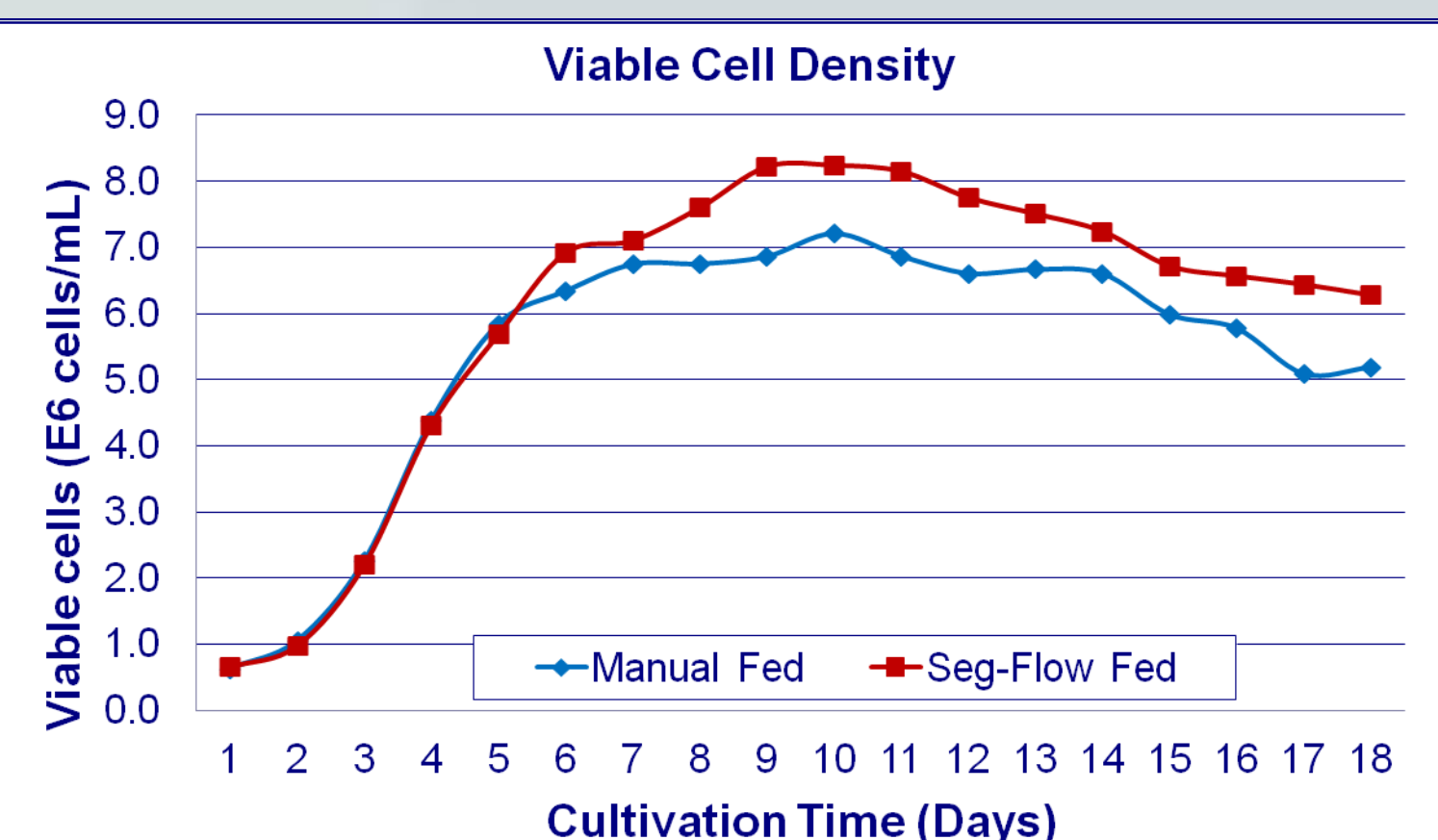
Automated On-line Sampling & Feed Control Benefits

• Eliminate sampling errors and operator-to-operator variability
• Eliminate weekend and evening manual sampling
• Enhance process monitoring through integrated analytical approach
• Increase process knowledge through real-time analysis
• Data acquisition and analysis integration
• Reduce process development time
• Improve process control and performance
• More consistent process output (product quantity and quality)
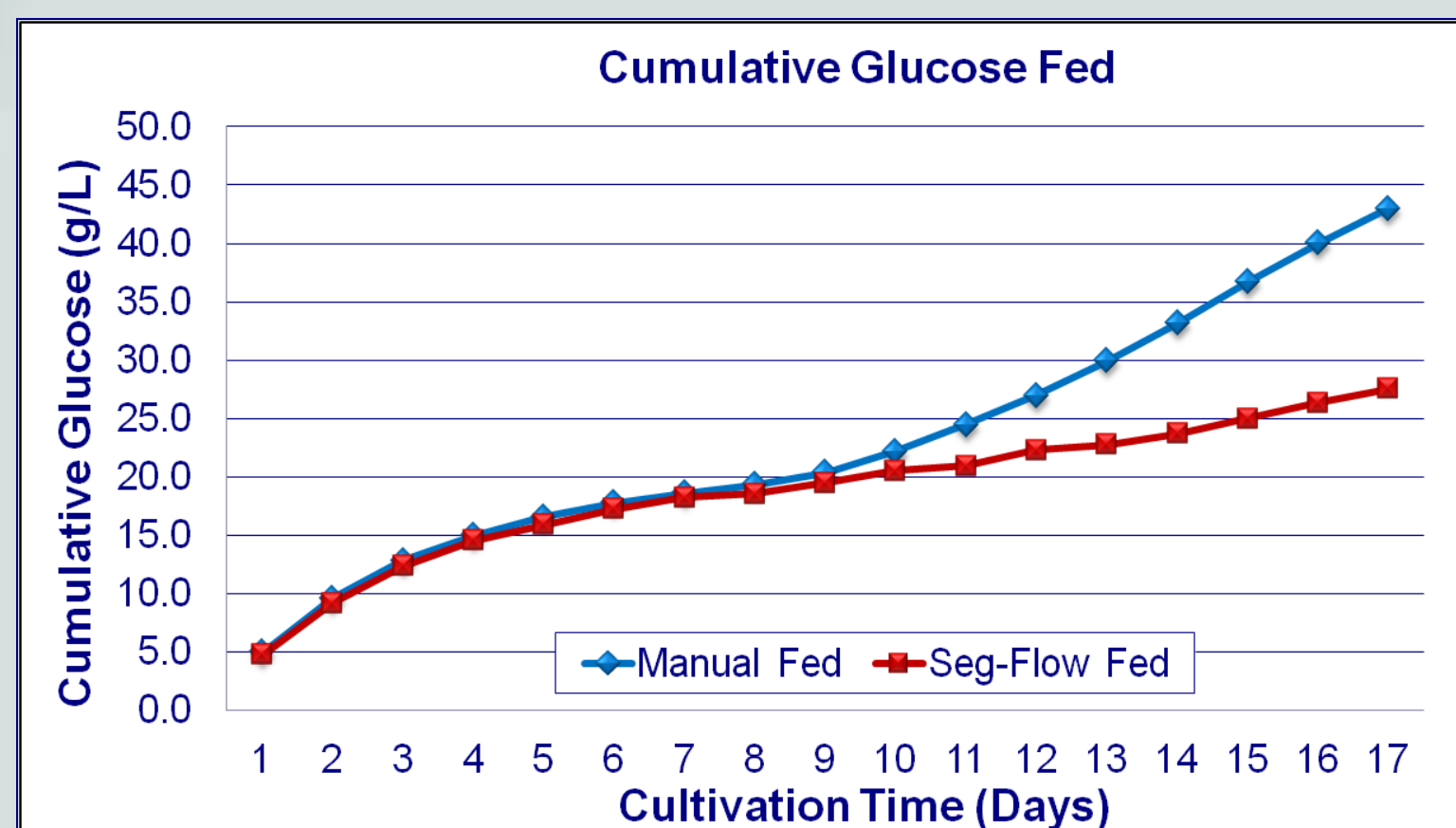
SEG-FLOW® Capabilities

• Withdraw cell-free and/or cell-containing samples from up to 8 bioreactors
• Integrated analysis and sample collection with up to 4 analyzers/fraction collectors
• Simultaneous online monitoring of nutrients, metabolites and cell growth/viability
• Provides centralized data acquisition for integrated analyzers
• Communicates analytical data to any bioreactor/fermenter SCADA system via OPC
• Highly flexible system with multiple bioreactor, analytical and feed pump interfaces
• Controls up to 2 pumps per vessel for various nutrient feed control options
• Remotely monitor and control sampling, feeding, and data

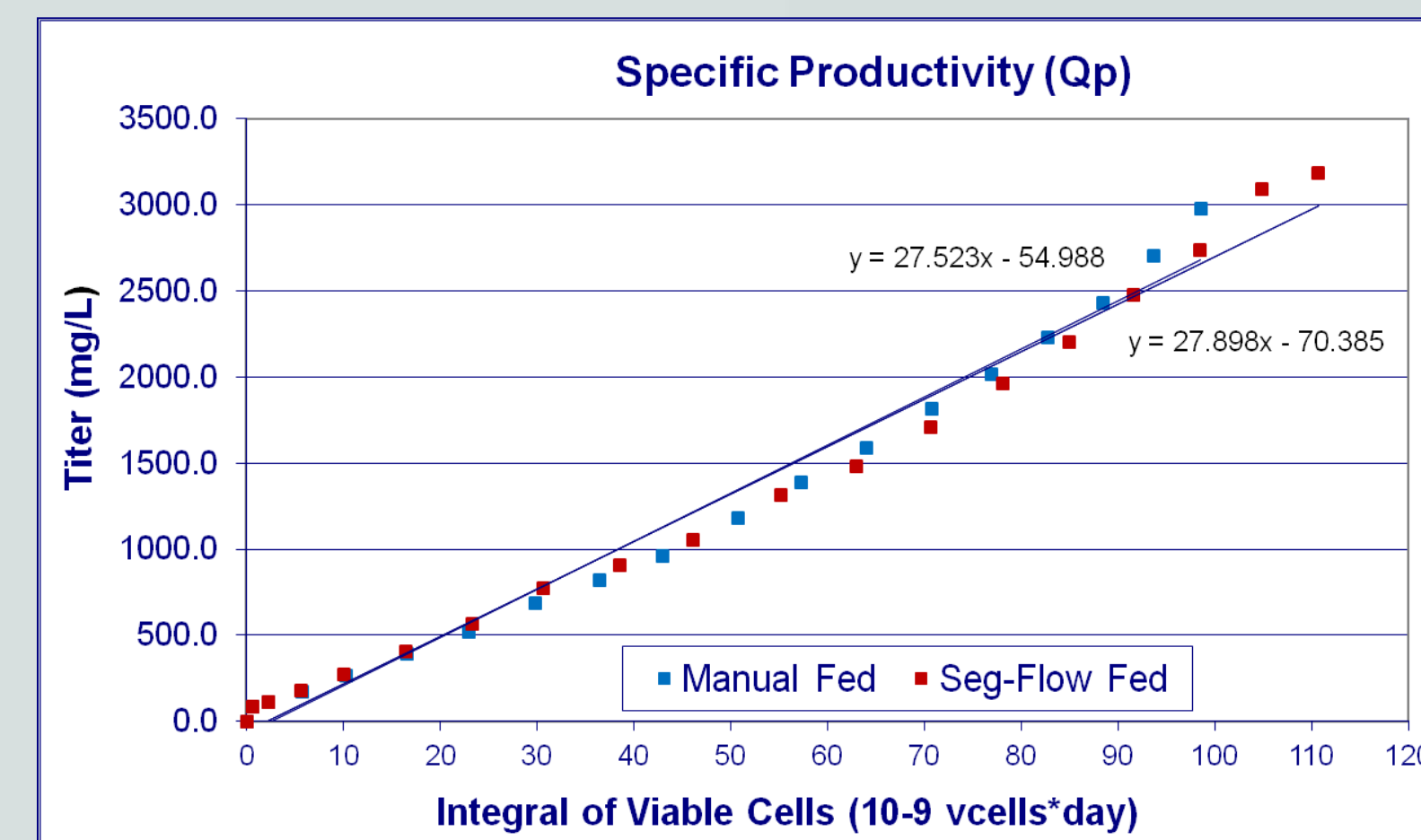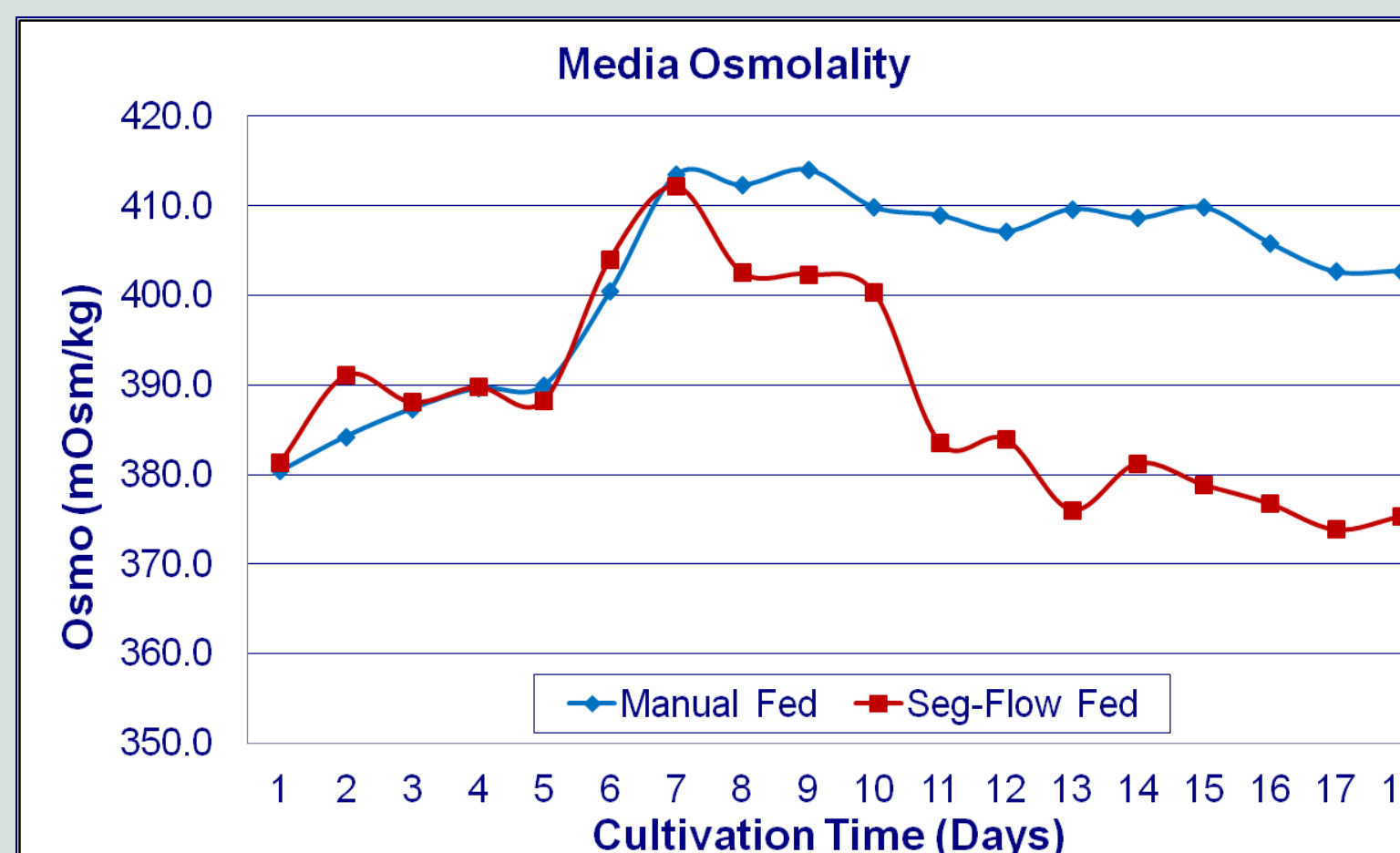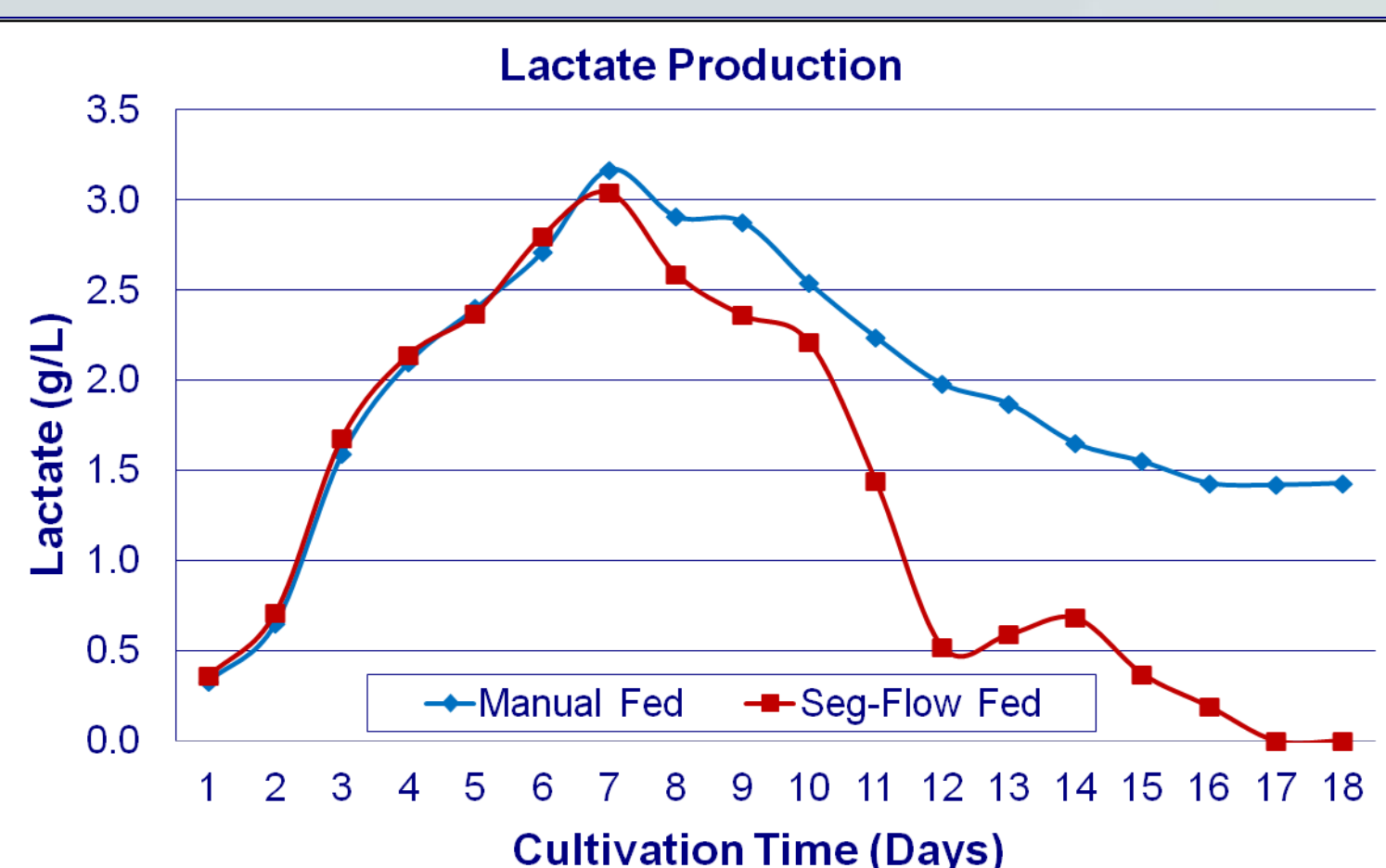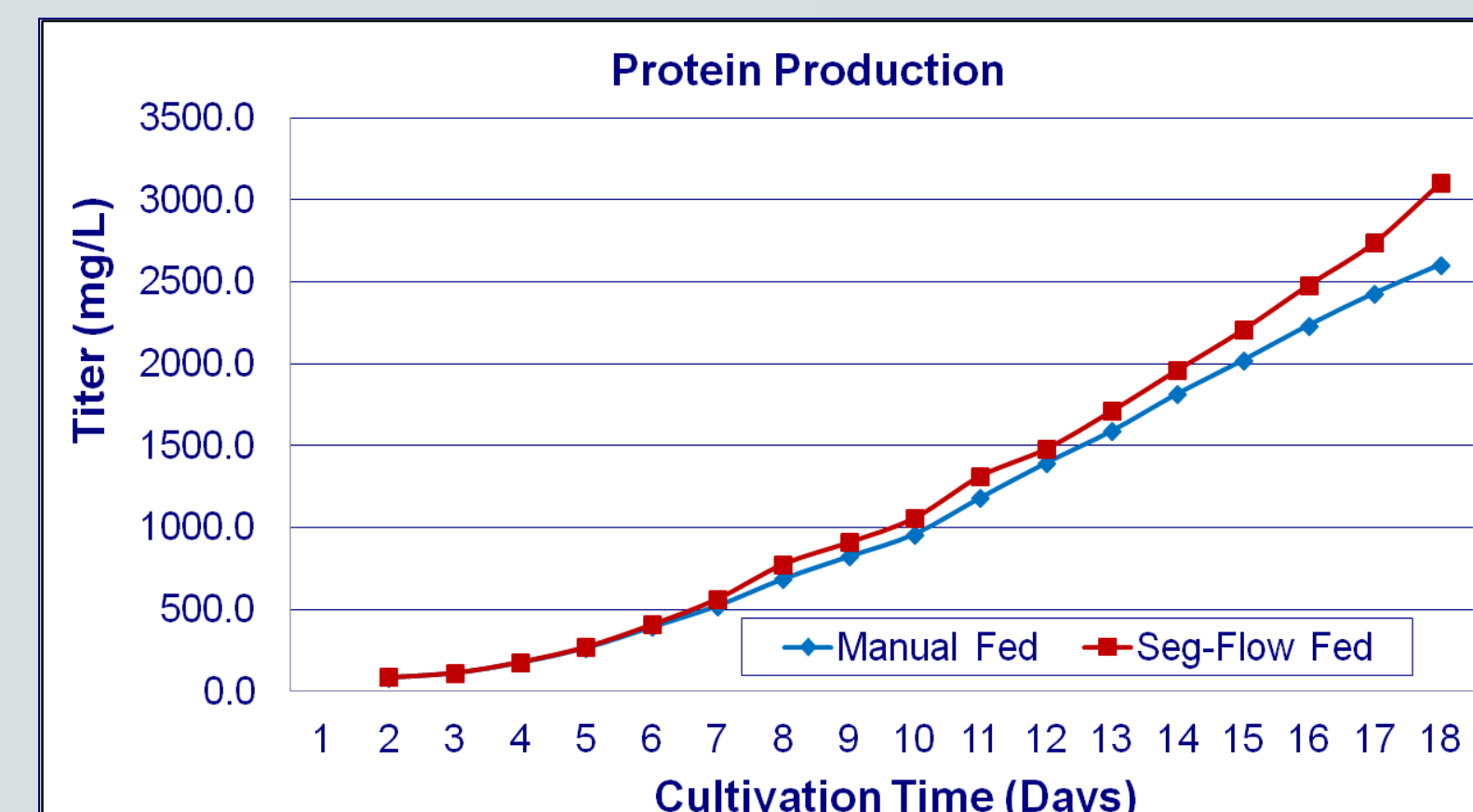## SEG-FLOW® Impact on Cell Culture Performance

Cell Growth & Metabolism

Viable Cell Density
(graph: Viable cells (E6 cells/mL) vs Cultivation Time (Days); Manual Fed, Seg-Flow Fed)

Lactate Production
(graph: Lactate (g/L) vs Cultivation Time (Days); Manual Fed, Seg-Flow Fed)

• Peak and harvest VCC's increased 1 x E6 cells/ml in Seg-Flow fed culture
• Lower lactate production exhibited in Seg-Flow fed culture

Media Glucose & Osmolality

Cumulative Glucose Fed
(graph: Cumulative Glucose (g/L) vs Cultivation Time (Days); Manual Fed, Seg-Flow Fed)

Media Osmolality
(graph: Osmo (mOsm/kg) vs Cultivation Time (Days); Manual Fed, Seg-Flow Fed)

• Reduced glucose requirement observed for Seg-Flow fed culture as evidenced by less cumulative glucose fed
• Media osmolality reduced ≥ 6% from day 8 through harvest resulting in base addition reduction

Product Titer & Specific Productivity

Protein Production
(graph: Titer (mg/L) vs Cultivation Time (Days); Manual Fed, Seg-Flow Fed)

Specific Productivity (Qp)
(graph: Titer (mg/L) vs Integral of Viable Cells (10-9 vcells*day); Manual Fed, Seg-Flow Fed)
$y = 27.523x - 54.988$
$y = 27.898x - 70.385$

• Recombinant protein production increased 16% in Seg-Flow fed culture
• Comparable specific productivity for both cultures; protein production increase due to increased cell mass

Conclusions

▪ **Improved CHO cell culture process performance achieved using the SEG-FLOW® automated on-line sampling and feed control system**

▪ **Automated on-line monitoring and feed control of glucose resulted in:**
  ▪ **Greater carbon conversion efficiency**
  ▪ **Increased cell density and product titer**
  ▪ **Reduced lactate production and lower media osmolality**

▪ **Increased process knowledge and understanding achieved through real-time analysis**

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