

# Automated On-line Sampling and Nutrient Monitoring for Characterizing Glucose

## Consumption of Microbial Fermentation Cultures

William Miller<sup>1</sup>, Cris Blattner<sup>2</sup>, David Frisch<sup>2</sup>, Hyunsic Choi<sup>2</sup>, Dmitriy Shevchenko<sup>2</sup>, Frederick R. Blattner<sup>2</sup> & Michael Biksacky<sup>1</sup>

<sup>1</sup> Flownamics, Inc., Madison, WI, <sup>2</sup> Scarab Genomics, LLC, Madison, WI



### Abstract

A SegFlow® automated on-line vessel sampling system (SegFlow System) was evaluated for characterizing real-time glucose consumption of microbial fermentation cultures used for protein biotherapeutic production. High cell density fermentation processes employing host strains of Scarab Genomics' Clean Genome® *E. coli* were conducted at 10L working volume using a biphasic approach. Cultures were grown to a specific density in minimal salts/glucose media during the batch phase. Upon reaching the targeted density, glucose was fed exponentially to control the culture growth rate at a specified  $\mu$  hr<sup>-1</sup> during the fed-batch phase. The SegFlow System, consisting of the SegFlow autosampling module, a YSI® Biochemistry Analyzer and a fraction collector, provided real-time analysis of media glucose concentrations as well as programmed time-point sampling for manual glucose analysis and additional off-line analytics. Automated and manual glucose analyses were compared to ensure fidelity of the results generated by the SegFlow System. The SegFlow System provided an automated, real-time solution for monitoring glucose concentrations and, in turn, characterizing glucose consumption of *E. coli* fermentation cultures. Results from the SegFlow System's automated glucose analytical method and the manual technique were comparable as well.

### Experimental Plan

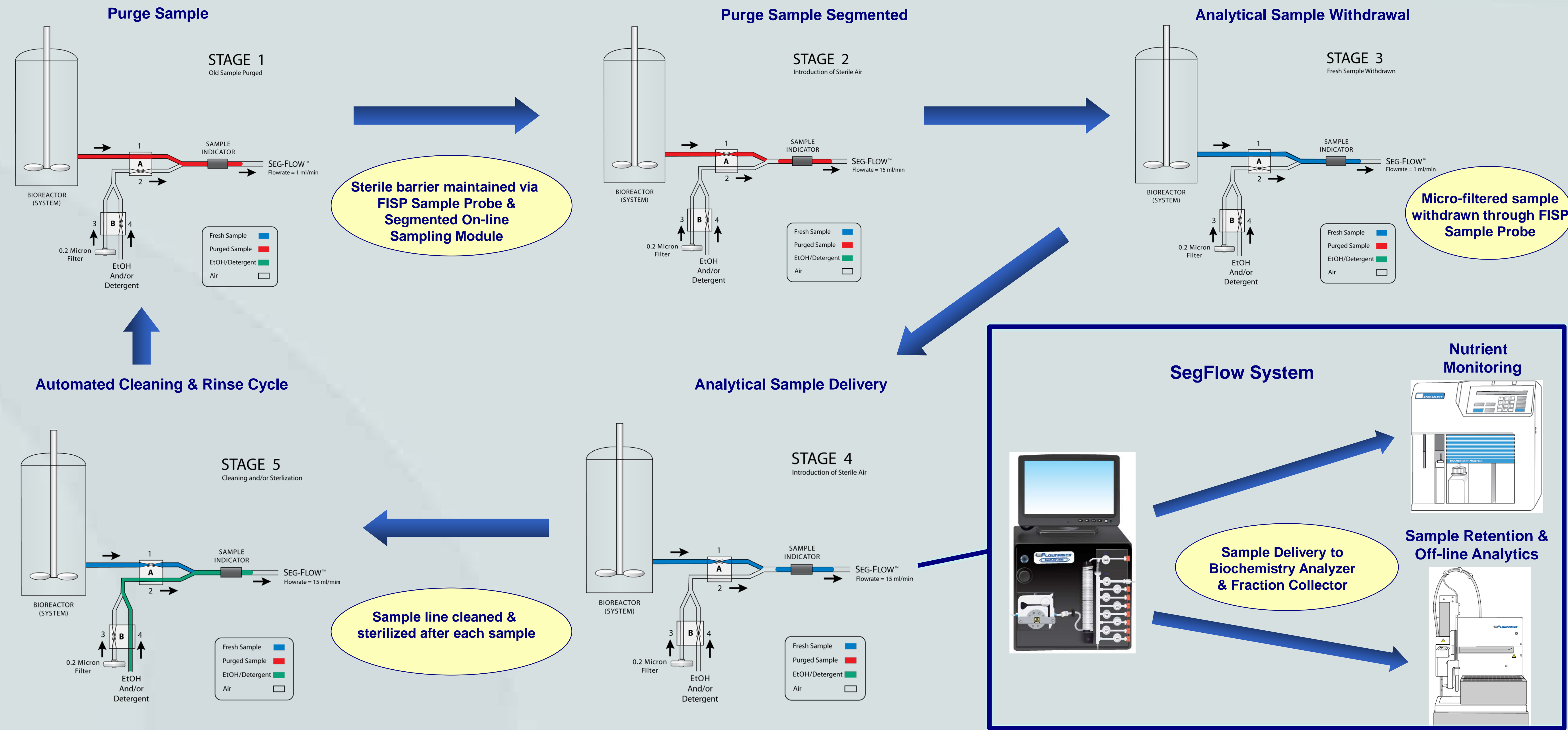
#### Rationale:

- Implementation of SegFlow automated process analytical technology (PAT) should provide rapid characterization of Clean Genome *E. coli* glucose metabolism.
- Comparability for both automated and manual glucose analytical methods should be demonstrated.

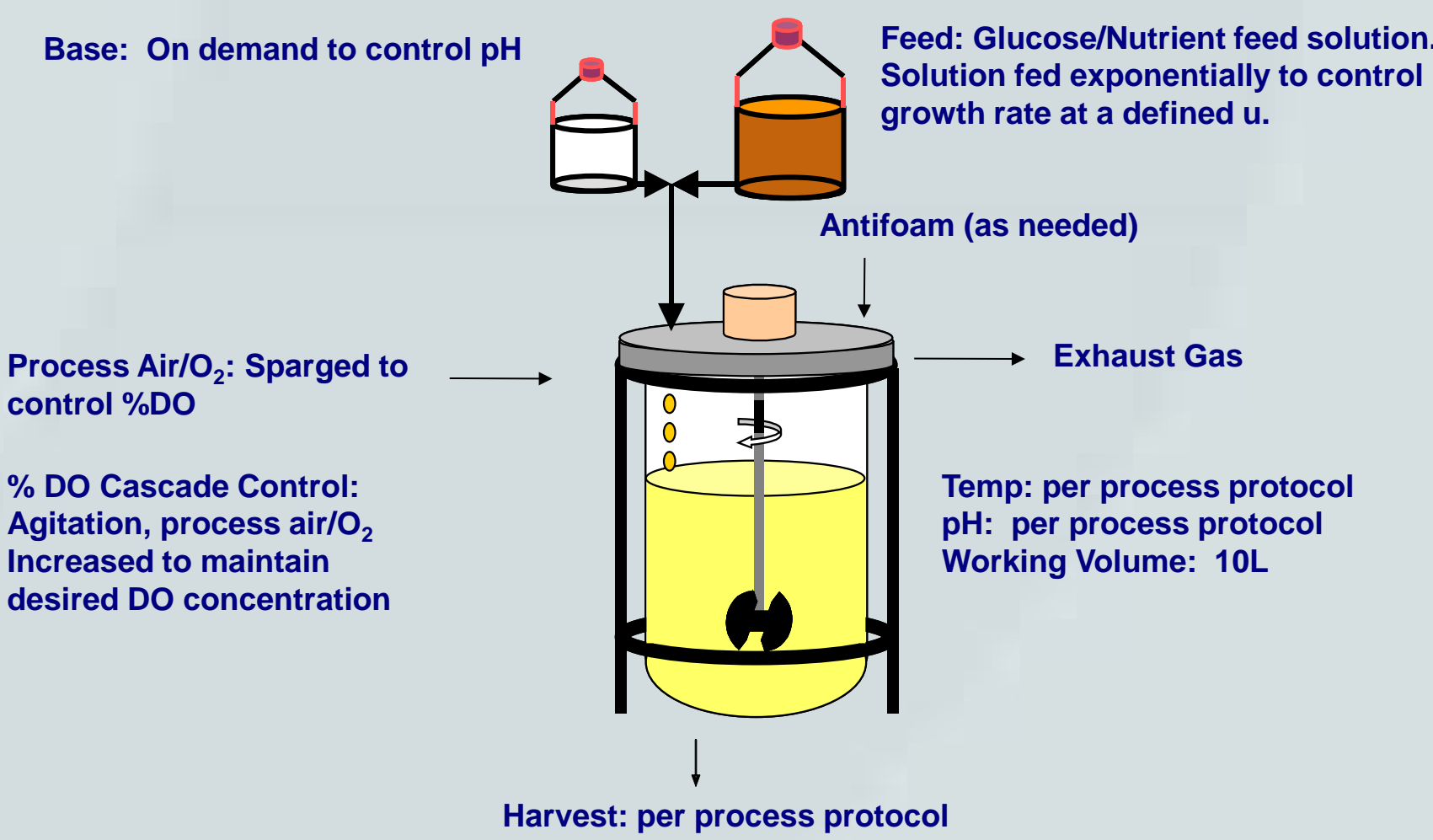
#### Evaluation:

- Evaluate the effectiveness of the SegFlow automated, on-line sampling system as a process analytical technology (PAT) for fermentation culture glucose monitoring as compared to an existing off-line glucose analytical method.

### Automated On-line Sampling & Nutrient Monitoring



### Fermentation Process



### Process Analytics

#### On-Line – cell-free samples

- SegFlow - automated online sampling - glucose analysis & fractions
- FISP Sample Probe – fermenter cell-free sampling
- YSI 2700 Biochemistry Analyzer – glucose analysis
- Flow Fraction 400™ Fraction Collector – manual glucose samples

#### Off-line – cell containing samples

- Megazyme Enzymatic Assay Kit /UV Spectrophotometer – acetate
- Spectrophotometer – cell density (OD)

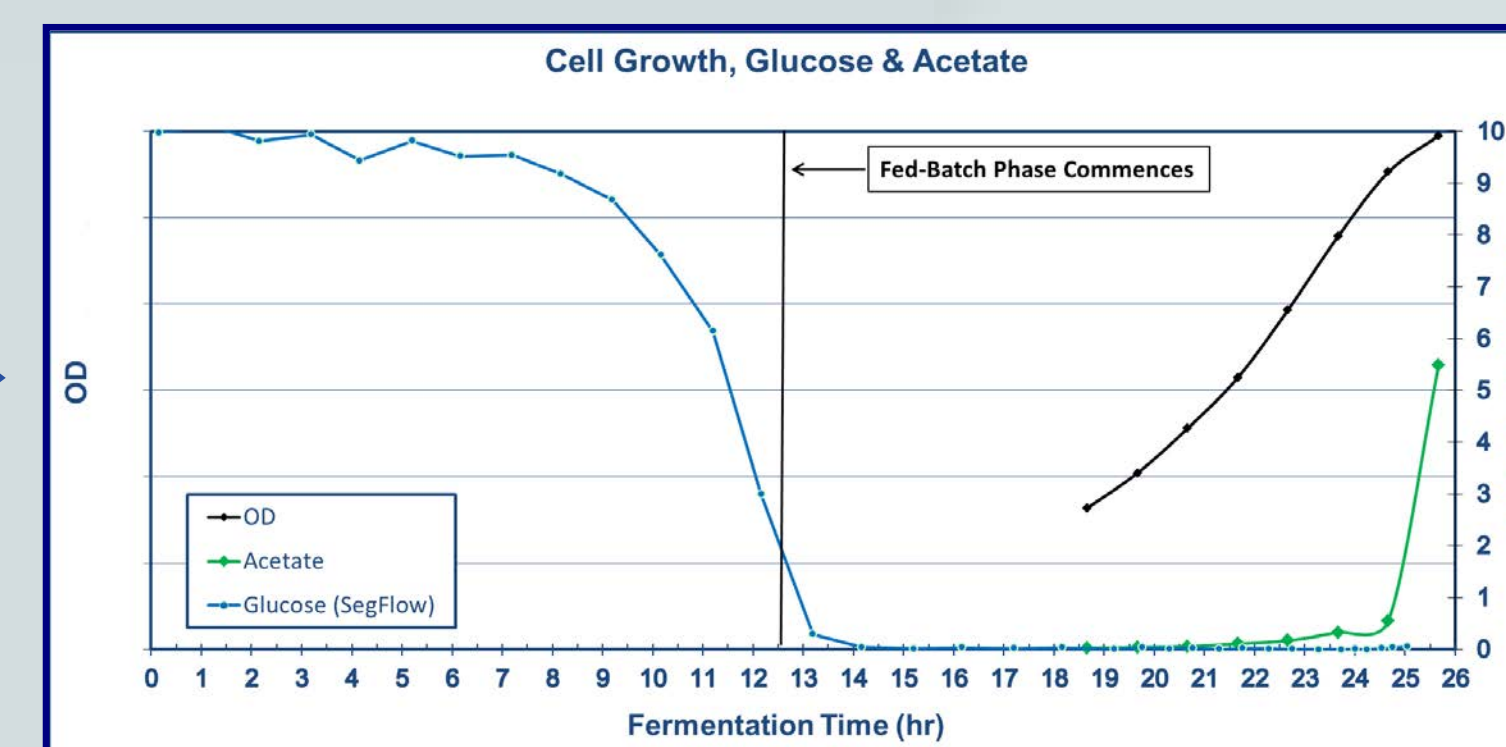
### SegFlow System



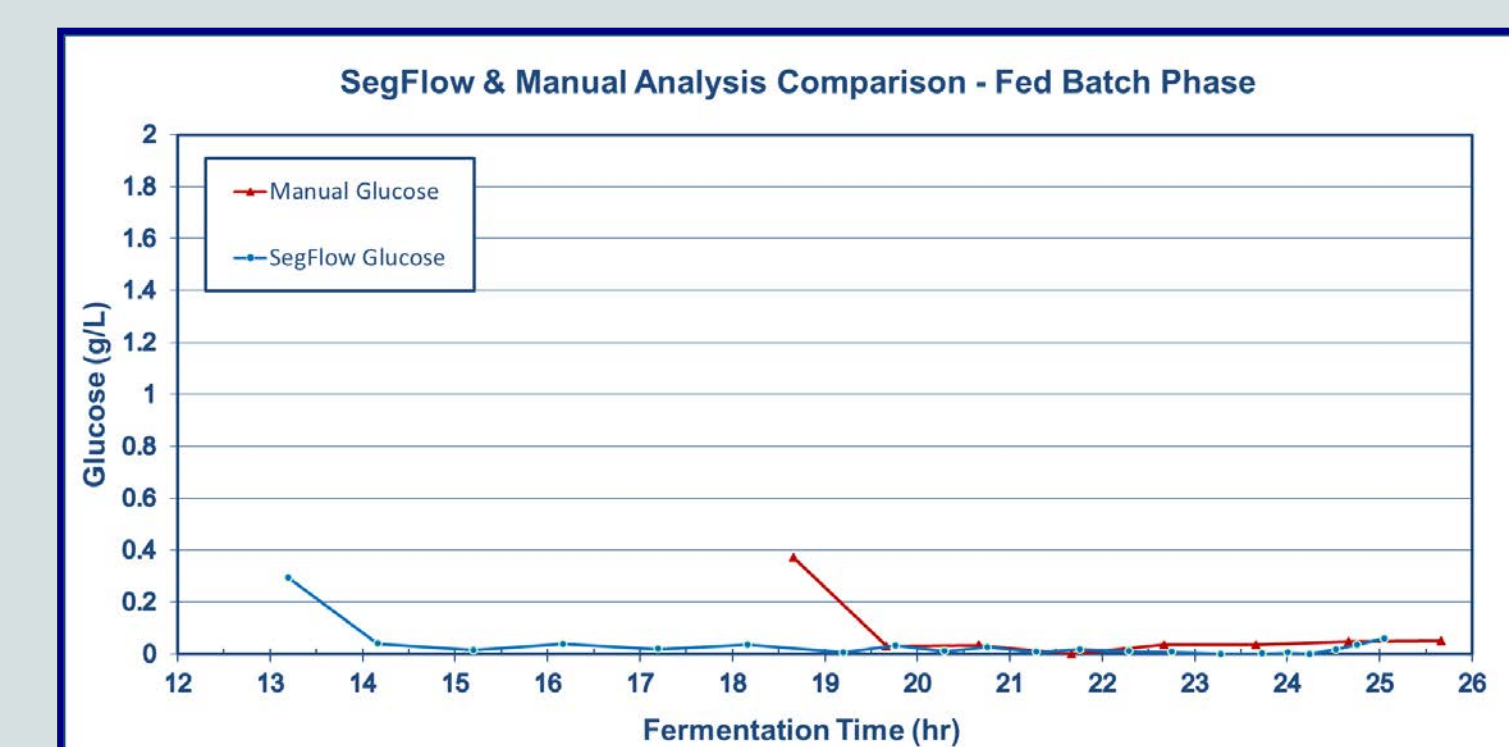
### Fermentation Culture Performance Data

#### Clean Genome Strain A

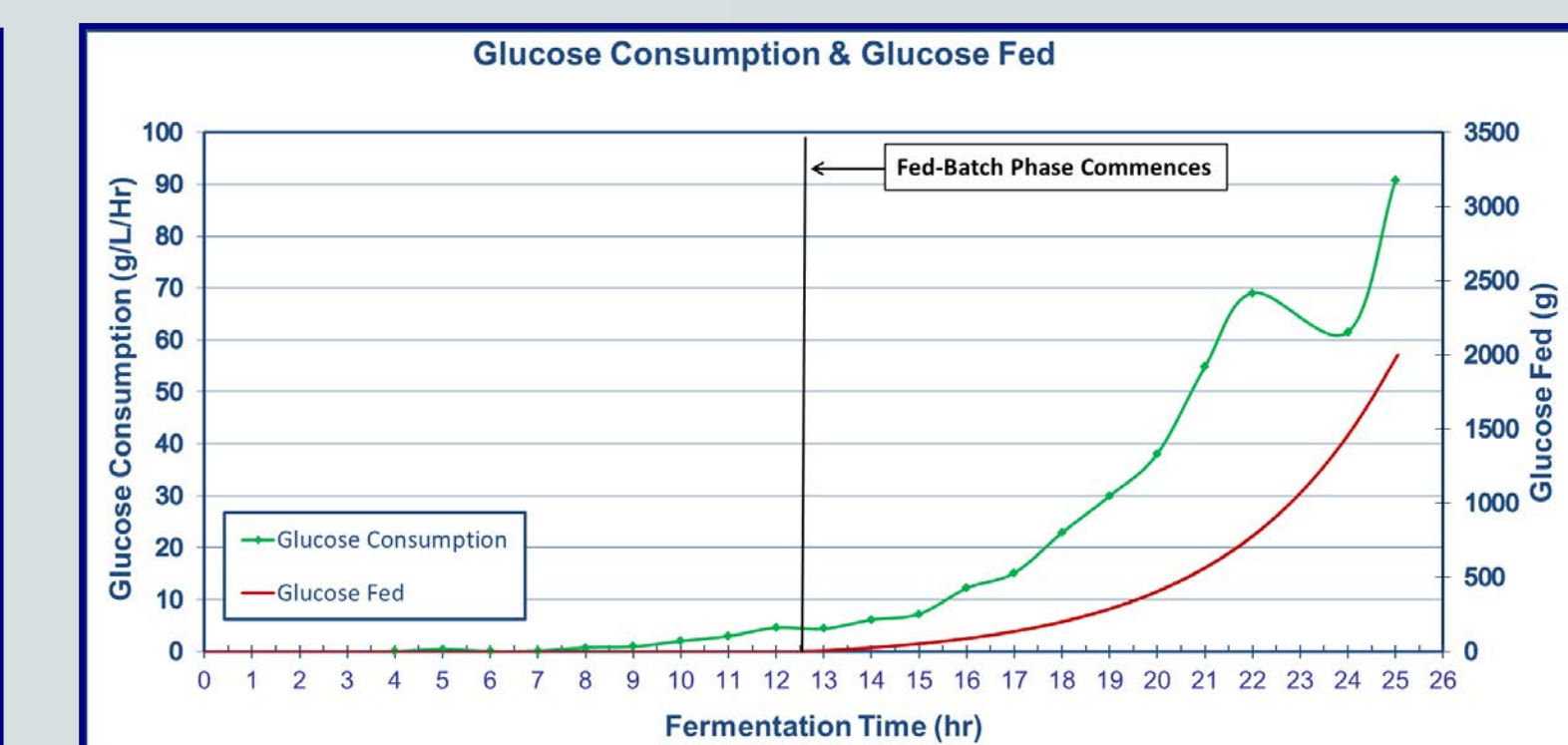
#### Cell Growth & Metabolism



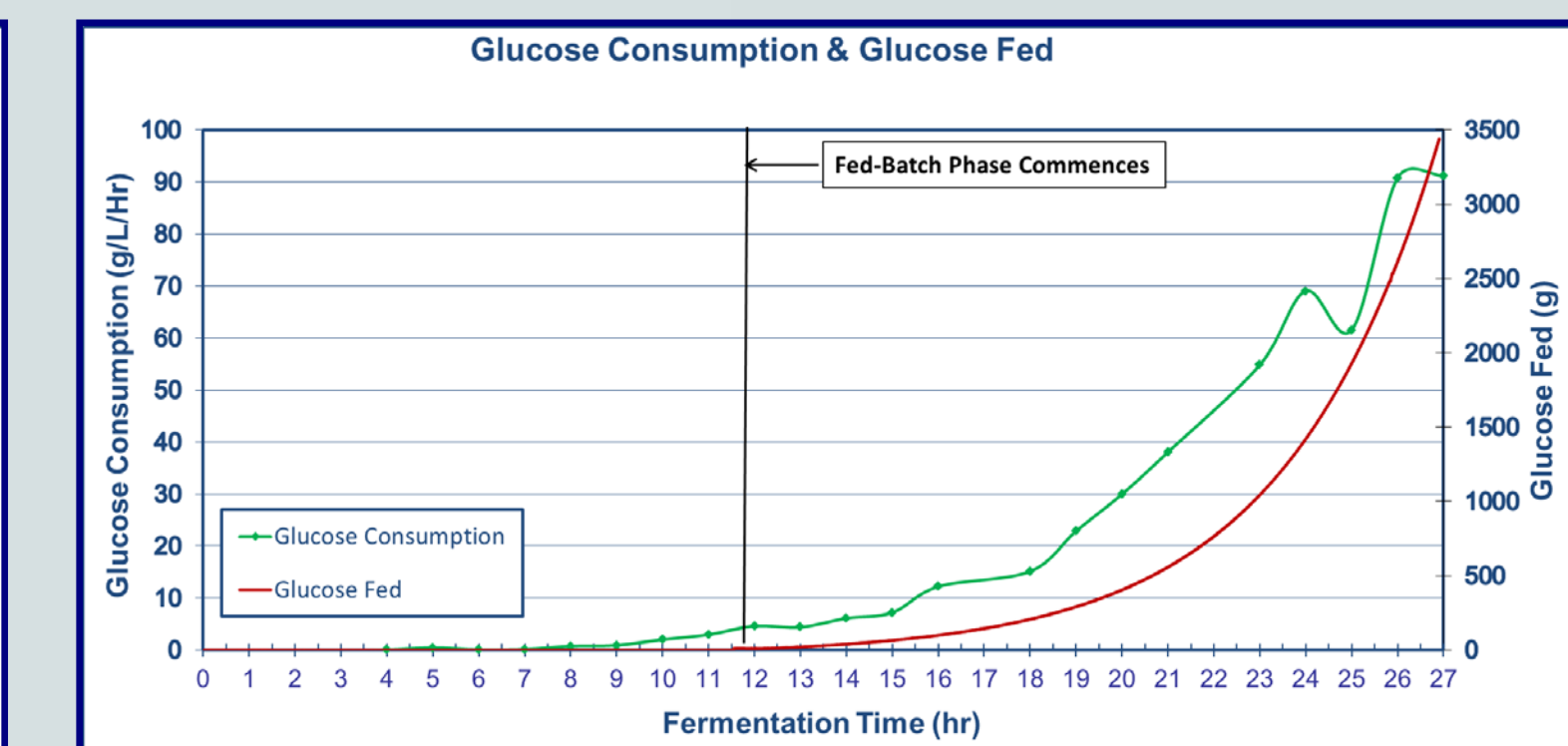
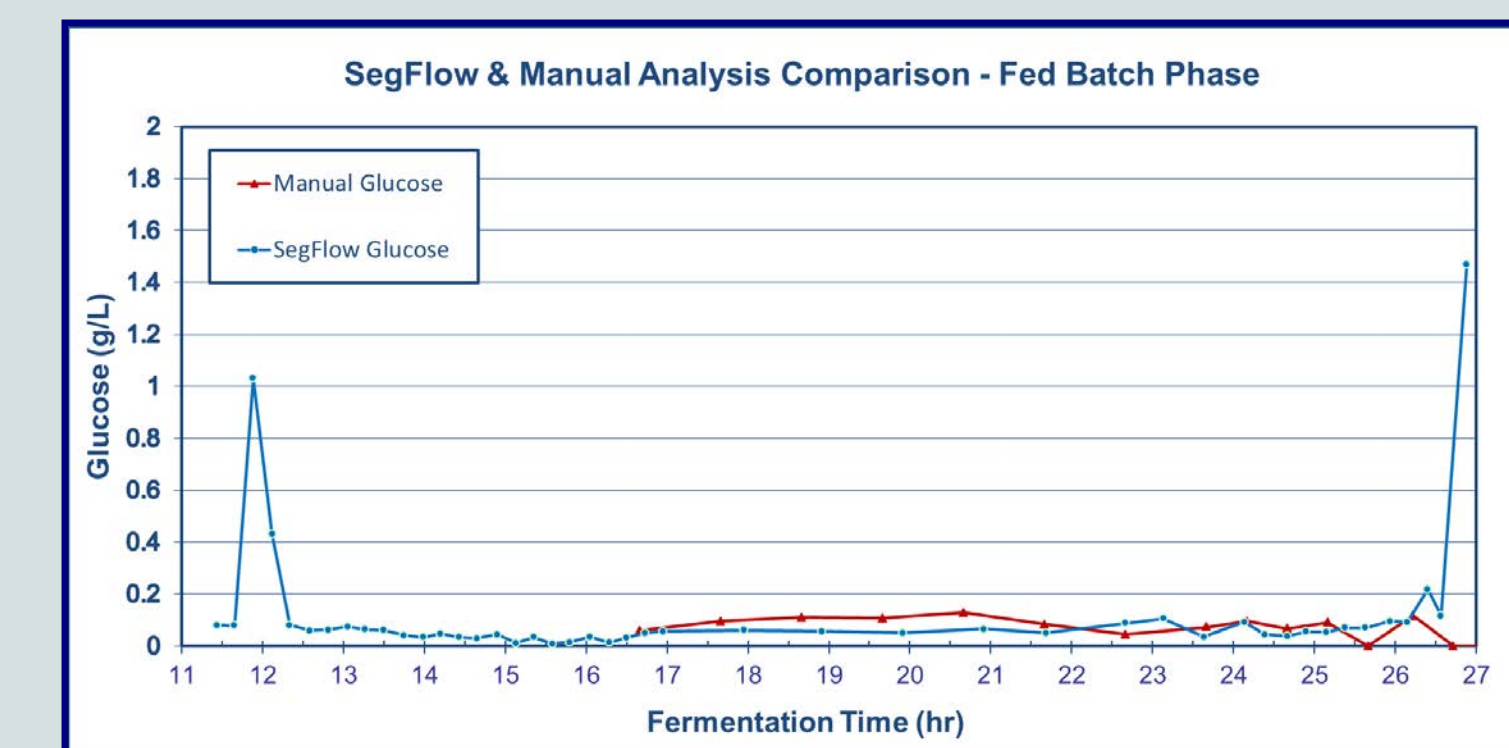
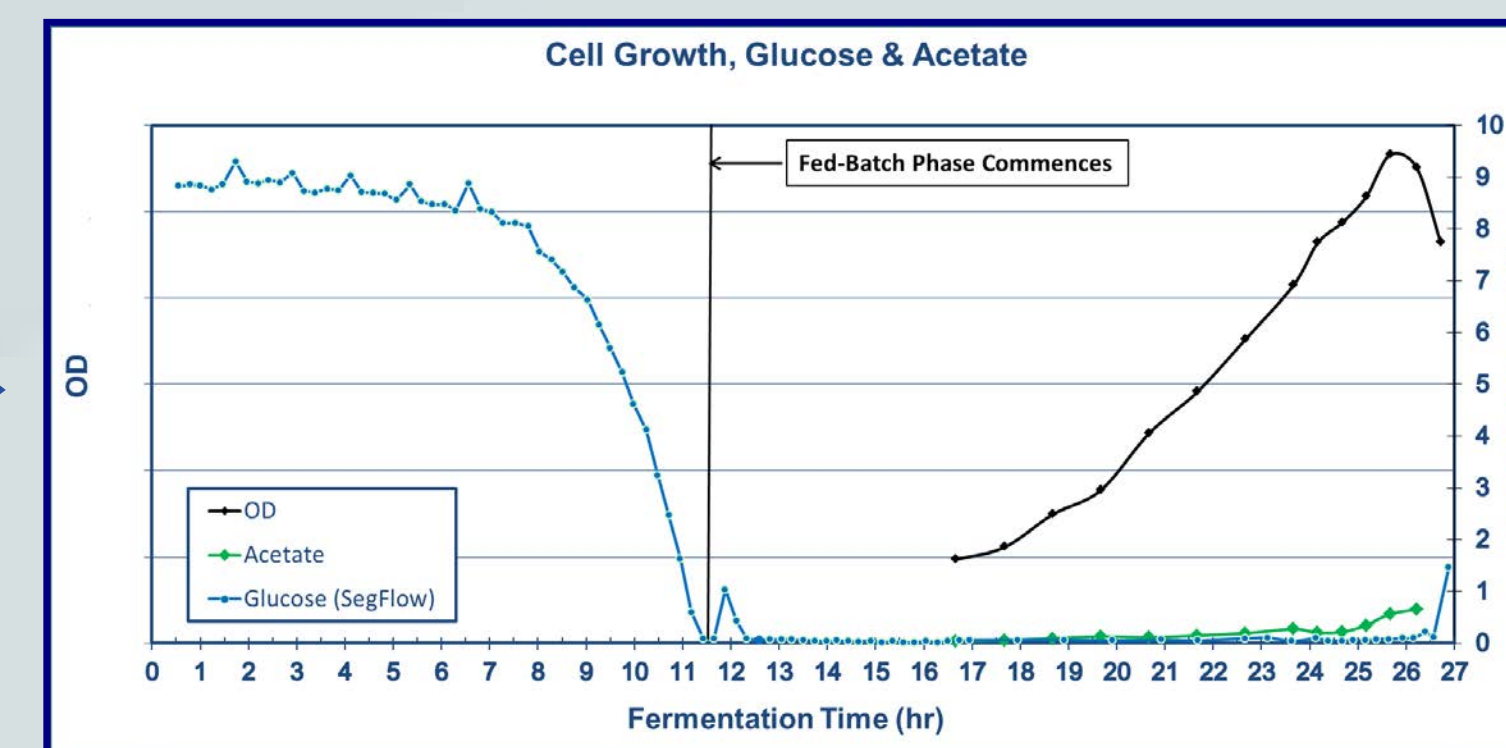
#### Glucose Analysis Comparison



#### Glucose Consumption & Glucose Fed



#### Clean Genome Strain B



- SegFlow system provided comprehensive glucose concentration profile throughout process duration
- Metabolic shift in glucose metabolism identified in real-time at end of fed-batch phase (Strain B)

- Same YSI 2700 Biochemistry analyzer used for comparing manual and automated methods
- Automated and manual glucose analyses comparable throughout fed-batch phase

- Characterization of glucose consumption profile expedited with SegFlow system
- High cell density cultures exhibit high glucose uptake rates

### Conclusions

- SegFlow System provided an automated, on-line PAT solution for real-time nutrient monitoring of fermentation cultures
- Rapid characterization of fermentation culture glucose consumption realized
- Cellular metabolic change for Clean Genome *E. coli* identified in real-time through use of the SegFlow System
- Comparability demonstrated for both on-line and off-line glucose analytical methods

### Acknowledgements

Flownamics, Inc.

- Jayson Preston
- Ashley Fisher

Scarab Genomics, LLC

- Heather Kirkpatrick



3025 S. Stoughton Road Phone: 608-240-1604  
Madison, WI 53716 Toll-Free: 800-932-6989  
www.flownamics.com