Application of SEG-FLOW™ System for Cell Culture Process Development

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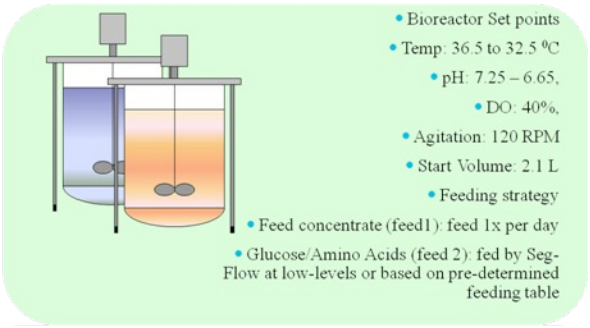
Summary

SEG-FLOW™ is an automated sampling system with feed-back control capability. It can be programmed to sample up to eight bioreactors and send cell-free samples to four analyzers/collectors. It also has an internal web server to enable remote access.

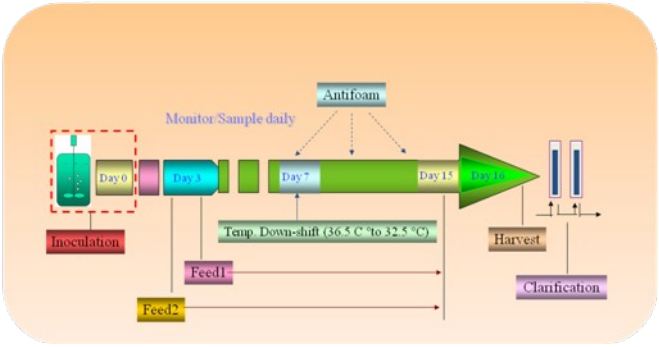
It is well known that glucose plays an important role in mammalian cell culture as carbon and energy sources. Therefore, it is important to understand the metabolism of glucose in order to develop strategies to maximize recombinant protein production and control product quality. In the present study, experiment was designed to evaluate the impact of programmed feeding on culture performance by SEG-FLOW and YSI 2700 interfaced with bioreactors. The automatic feed control resulted in smaller change intervals of feed medium as compared to daily manual bolus feeding. Our results showed that the application of SEG-FLOW system could potentially reduce labor, improve bioreactor efficiencies. In combination with optimized cell culture media and feeds, continuous feeding control could result in condition at which higher total protein production could be achieved, leading to more efficient protein manufacturing as compared to that with generic fermentation process. Product quality was examined and reverse phase HPLC data are presented.



Materials & Methods

Cell culture

Recombinant CHO cells expressing a fusion protein were maintained in a proprietary protein-free medium (Centocor, Inc.) and cultivated in stirred-tank bioreactors (Applikon) with a fed-batch mode. pH, relative oxygen saturation and temperature were controlled. Cultures were fed daily after day 3 post-inoculation with a proprietary nutrient concentrate (Centocor, Inc.). A glucose containing amino acid cocktail was also fed by either Seg-Flow (controlled semi-continuous feeding to maintain glucose set point) or the operators (daily bolus feeding). cells were cultivated at conditions of temperature shift profiles, and the temperature was shifted down from 36.5 to 32.5 °C after 6 days of cultivation.



Assessment of cell growth, protein productivity

Cell density and viability was determined by Cedex automatic cell counter (Innovatis). Cellular metabolic profiles were analyzed by NOVA bioanalyzer (NOVA Biomedical) and blood gas analyzer (BGA). For protein productivity, a protein-A based HPLC system (model 1100, Agilent Technologies) was employed to make this determination.

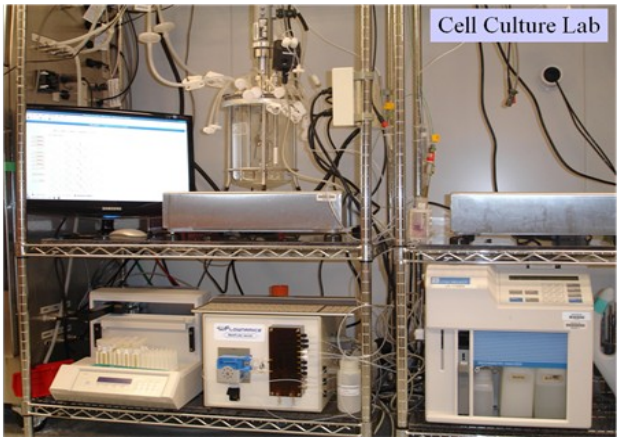
Analytical assessment

Protein aggregation was measured by size exclusion chromatography (Agilent Technologies). Agilent 1200 Reverse Phase HPLC (Agilent Technologies) was used for clipping profiling. Intact mass and Oligo map analysis will also be performed.

SEG-FLOW™

Features

- Sample size as low as 0.25 - 1 ml
- Rapid and accurate sampling
- Withdraw samples using FISP® Probes
- Feed control
- Internal web server
- Low cost and small foot print
- *(Sample prep)*



Experimental Results

Cell Growth &Productivity

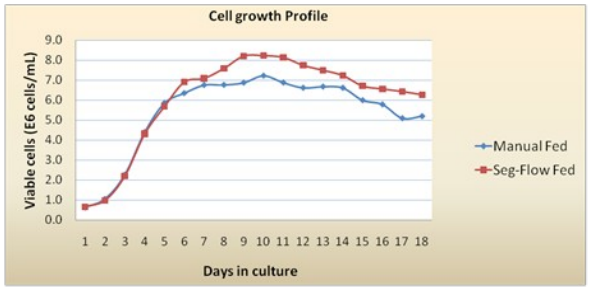


Fig. 1. **Cell growth profiles in fed-batch bioreactors.** CHO cells expressing a fusion protein were cultivated in fed-batch bioreactors under two different feeding strategies as described in the material and methods. Viable cell density and viability were determined by Nova bioanalyzer.

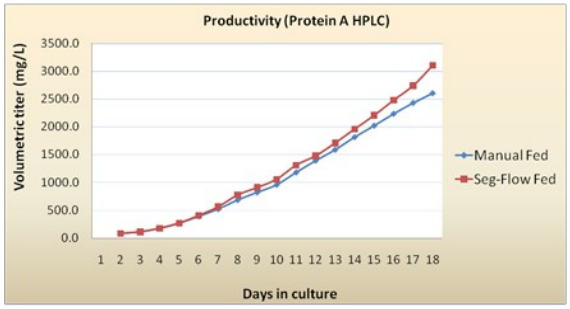


Fig. 2. **Impact of feeding strategies on product titer.** As compared with bolus feeding, feeding with low levels of glucose (controlled by Seg-Flow) has increased titer.

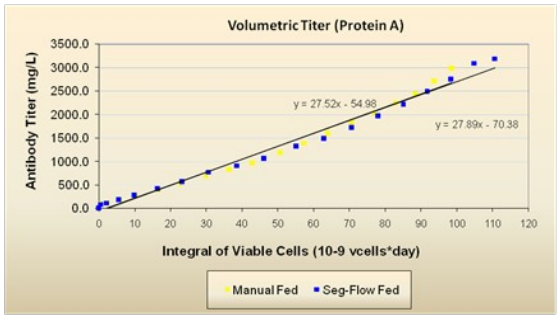


Fig. 3 **Impact of feeding strategies on specific titer.** As compared with bolus feeding, feeding at low levels of glucose (controlled by Seg-Flow) generated more cell mass and therefore show comparable specific productivity (Qp).

Culture Metabolites

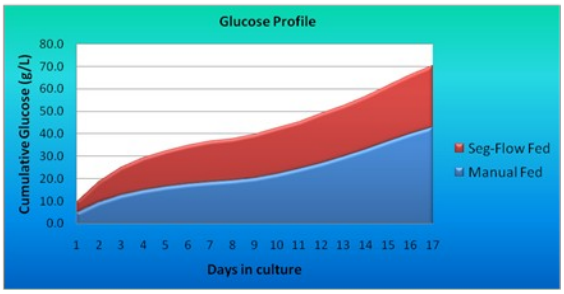


Fig. 4. **Glucose feeding Profiles.** As compared with bolus feeding, programmed Seg-Flow system is able to deliver significant less amount of glucose to cell culture, but provide enough to maintain set-point concentration on a semi-continuous basis (e.g. every 5 hrs).

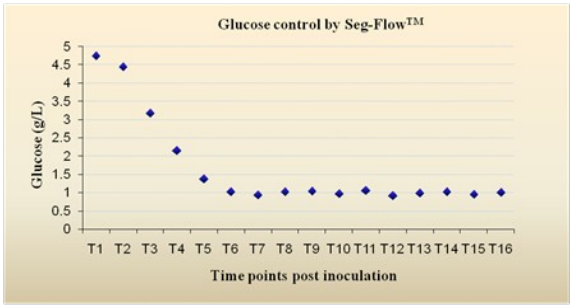


Fig. 5. **Glucose feeding Profiles.** Seg-Flow system is able to consistently deliver small amount of glucose as programmed to maintain cell culture glucose set point at low levels (e.g. ~ 0.9 g/L).

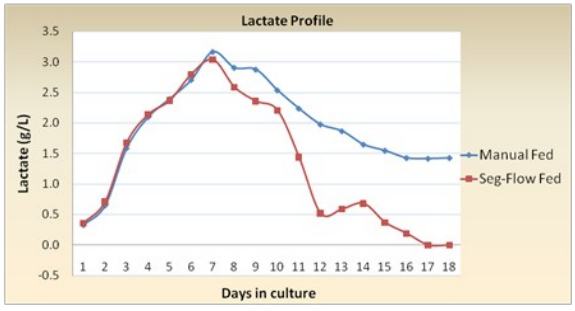


Fig. 6. **Impact of feeding strategies on Lactate metabolism.** As compared with bolus feeding, feeding with low levels of glucose (controlled by Seg-Flow) exhibited lower lactate levels during fermentation.

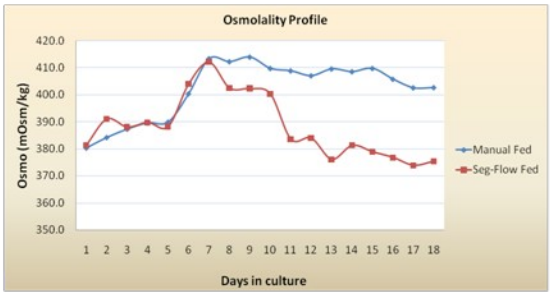


Fig. 7. **Impact of feeding strategies on Osmolality.** As compared with bolus feeding, semi-continuous feeding controlled by Seg-Flow exhibited lower Osmo level.

Analytical Data

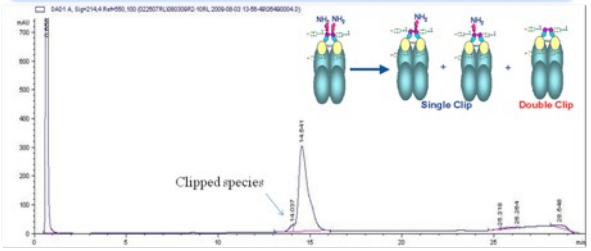


Fig. 8. **Impact of manual bolus feeding on product clipping.** Proteolytic clipping of product N-terminal (s) in fermentation samples were analyzed by Reverse-Phase (RP) HPLC method. Manual feeding condition showed ~ 3.6% total clip on day-11 post-inoculation.

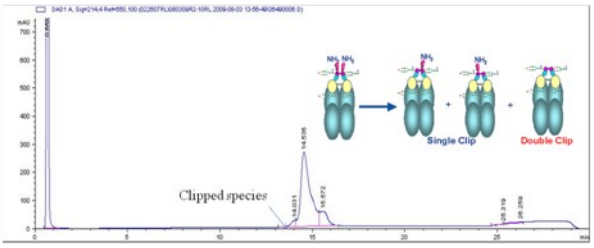


Figure 9: **Impact of Seg-Flow feeding strategy on product clipping.** RP-HPLC analysis showed that culture fed by Seg-Flow at low glucose levels gave similar total clip levels (~ 3.5%) on day-11. However, product glycosylation profile might be different from that of the manual bolus feeding for this particular molecule.

Conclusions-Ongoing work

❑ Seg-Flow system is easy to operate and it can consistently provide feed-back control and maintain target nutrient (e.g. glucose) levels at set-point(s) during fermentation. It performs automatic feeding and takes cell-free samples for up to eight bioreactors. Seg-Flow is also capable of sending cell-free samples to offline analyzers and/or fraction collector. These characteristics in particular useful for cell culture lab weekend sampling/feeding support and it may help to reduce labor.

❑ Cell Culture fed with Seg-Flow showed lower lactate and Osmo levels, which could be beneficial to cell growth and protein production in CHO system. Enhanced productivity (e.g. 15 – 20% higher) was observed in the current study controlled and fed by Seg-Flow.

❑ Manual feeding and feeding performed by Seg-Flow showed similar clipping levels and comparable aggregation profile (data not shown), however product glycosylation pattern might be different at different glucose levels as observed in this study by reverse-HPLC analysis. Therefore, Seg-Flow is also an useful tool for process optimization and product quality assessment.

❑ Future work: Perform feeding optimization to evaluate continuous feeding at higher glucose levels. Apply Seg-Flow system to cell culture with other CHO cell lines to examine feeding effect on productivity and product quality.

Acknowledgments

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