Automated On-Line Real Time Reactor Feedback Control

Integration of the Groton Automated Reactor Sampler, the DASGIP Bioreactor and the YSI Biochemistry Analyzer

I. Summary

Bioprocess monitoring and control of key analytes such as glucose, lactate, amino acids, and ammonia levels is critical to obtain optimal and consistently high productivity of a cell culture system. Improved productivity is critical to the development of high efficiency, economical production processes for therapeutic proteins and monoclonal antibodies. Despite the apparent value of feedback control systems in the biopharmaceutical industry there are few successful demonstrations. Here we present a complete, closed loop feedback control system by integrating a Groton Biosystems Automated Reactor Sample System with an YSI biochemistry analyzer and a DASGIP bioreactor control system in order to achieve a powerful, automated online, real time feedback control system for mammalian cell culture.

With real time sampling and feedback control we provide an effective and efficient tool for the optimization of mammalian cell culture bioprocess. This system can easily be adapted to other types of manufacturing systems for biologics, for example, microbial fermentation. In addition, this system would also promote the FDA Process Analytical Technology (PAT) initiative.

II. Materials and Methods

A Groton Biosystems Automated Reactor Sampler ("ARS-M") is connected to a 4 vessel DASGIP Parallel Bioreactor System ("PBS", Jülich, Germany) controlled by the DASGIP Control 4.0 OPC Edition Software ("DCS") and an YSI7100 Biochemistry Analyzer ("YSI", Yellow Springs, Ohio).

To illustrate and demonstrate the real time process feedback control scheme we used glucose as a cell culture test parameter. Initially, the bioreactors are filled with DMEM low glucose (1 g/L) cell culture medium (Sigma). After priming the ARS-M input and output paths (5mL each), a 3 mL sample was delivered automatically to the YSI7100 for analysis of glucose concentration. The YSI Biochemistry Analyzer then transmits automatically the glucose analytical data to Groton's ARS-M server where the data is stored. The DASGIP Control OPC client automatically polls for and retrieves new analytical data from the ARS-M OPC server. If the glucose concentration is lower than the set point, the DCS then responds by activating the appropriate system pump to deliver a calculated amount of glucose stock solution into the bioreactor to achieve the preset final concentration. Please refer to Figure 1 and 2 for a schematic and a photo of the set up of the systems.

The current Groton ARS-M series system is capable of connecting to and sampling from eight bioreactors. It can deliver the sample to as many as four analytical instruments such as YSI Biochemistry or NOVA Bioprofile™ Analyzers; Agilent HPLCs; and the Beckman Coulter ViCell™ or Innovatis Cedex™ cell viability monitors.

The DASGIP Parallel Bioreactor Systems can operate four, eight or sixteen bioreactors at the same time controlling pH, dissolved oxygen, temperature, agitation and liquid feeds independently for each reactor. DASGIP systems are equipped with high-accuracy miniature peristaltic pumps featuring variable speed drives. The flow rate of every pump can get user programmed to be controlled by a separate function based on online data, e.g. analytical data retrieved via OPC. Concurrent feedback control of multiple analytes for both nutrients and metabolites can be achieved.

The YSI7100 system has the capability to analyze up to 6 analytes.

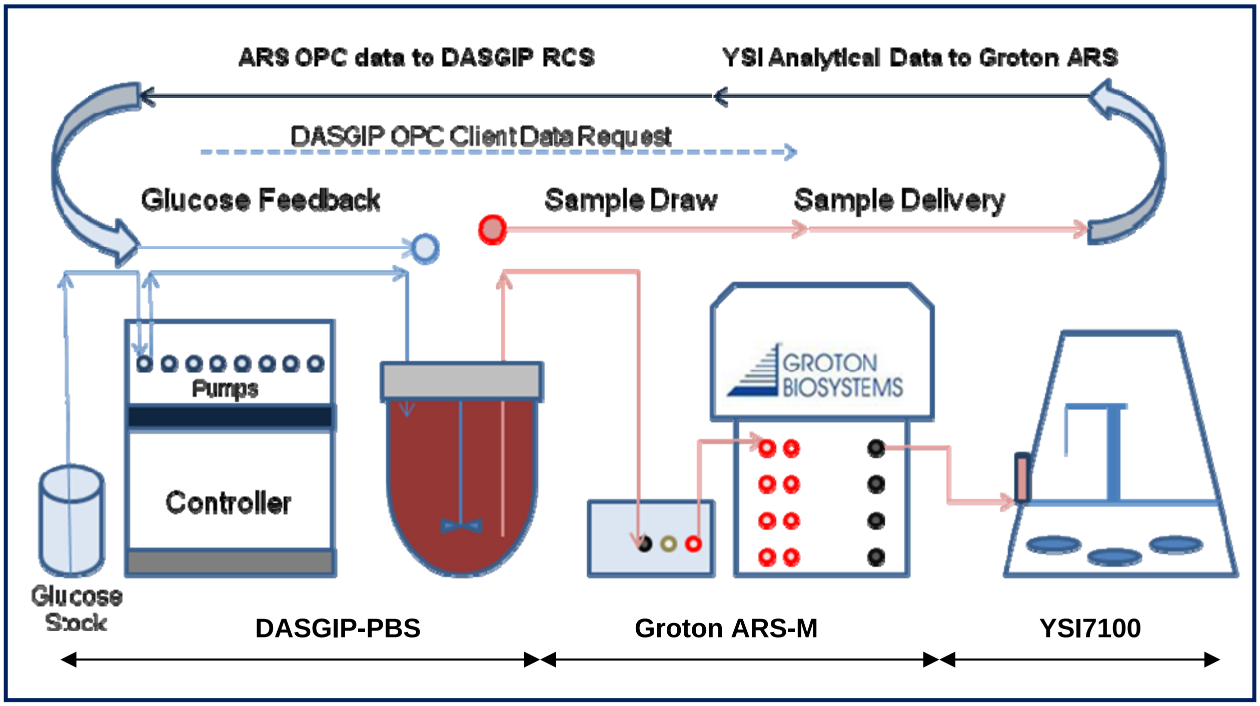

Figure 1: Automated Closed Loop Feedback Control

DASGIP AG ▪ Rudolf-Schulten-Straße 5 ▪ 52428 Jülich ▪ Germany
Tel: +49 2461/980-0 ▪ Fax: +49 2461/980-100 ▪ www.dasgip.com

DASGIP BioTools, LLC ▪ 151 Memorial Drive ▪ Shrewsbury, MA 01545
Tel: +1 508 842 69 00 ▪ Fax: +1 508 842 69 01 ▪ www.dasgip.com

Figure 2: DASGIP, Groton ARS-M, YSI 7100 Setup

III. Results and Data

A) Batch Glucose Feedback

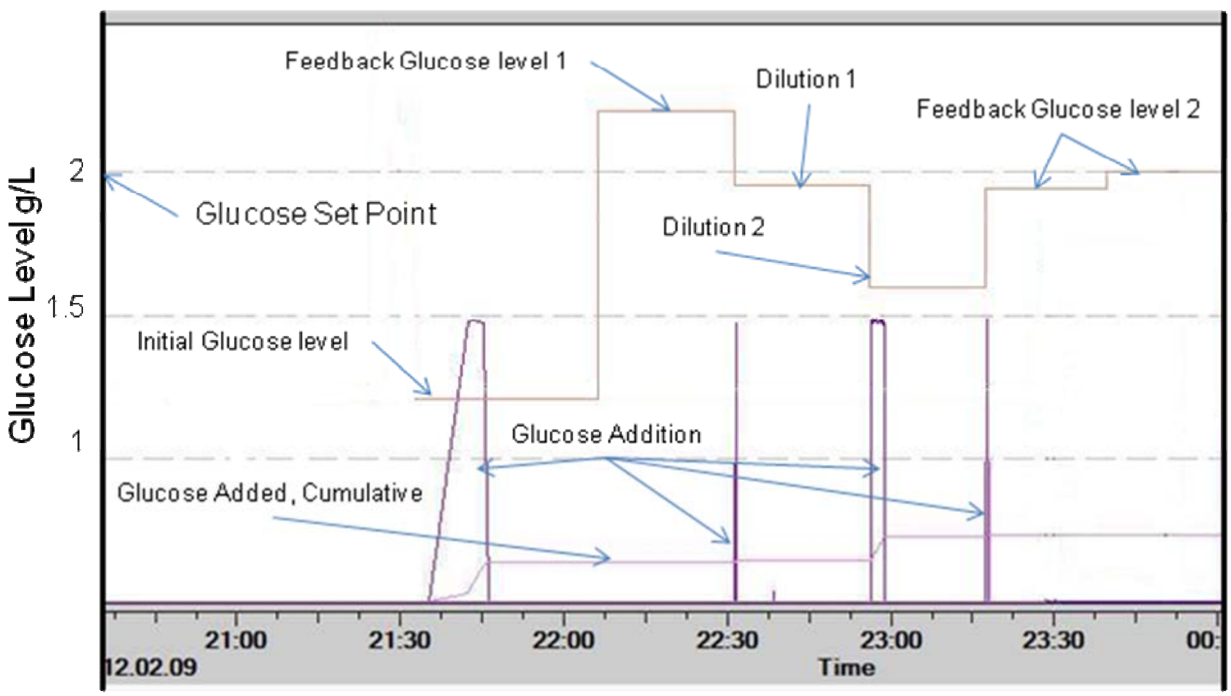

Figure 3: Batch Glucose Feedback Control

This experiment demonstrates the general feedback loop of the system. The glucose concentration in the bioreactor was programmed to adjust from 1 g/L to a preset level of 2 g/L through feedback mechanism of the system.

The initial volume of the medium was approximately 300 mL. A sample was drawn from Bioreactor 1 by the ARS-M and delivered to the YSI7100 for glucose analysis. The glucose concentration of 1.068 g/L was reported to the ARS-M controller. The DASGIP controller acquired the data from Groton's ARS-M server. Since the value was lower than the set point of 2g/L, it instructed the glucose feed pump to pump glucose stock solution (100g/L) into bioreactor 1. The total volume of glucose stock solution pumped was 2.8 mL. A sample was then drawn automatically by the ARS-M to verify the glucose concentration which was determined to be 2.263 g/L. An incorrectly set value of medium volume in bioreactor 1 caused an overshoot to the set point in the first feed. However, it was corrected easily as shown in the next repeat experiment.

The medium was then manually diluted in two steps (Dilution 1 and 2 in figure 3) with 200mL of water in total to produce an new initial glucose level of 1.531 g/L. Through the ARS–DCS feedback loop mechanism 1.625 mL of Glucose stock solution was pumped into Bioreactor 1 to elevate the glucose concentration to 1.947 g/L as measured by the ARS-YSI on the next sample draw. This was slightly lower than the set point level of 2g/L; therefore, the DCS instructed the pump to feed glucose stock solution (0.0194 mL) into bioreactor 1 to achieve a final concentration of 2.013 g/L. Figure 3 showed the process of this experiment.

B) Stepwise Glucose Feedback

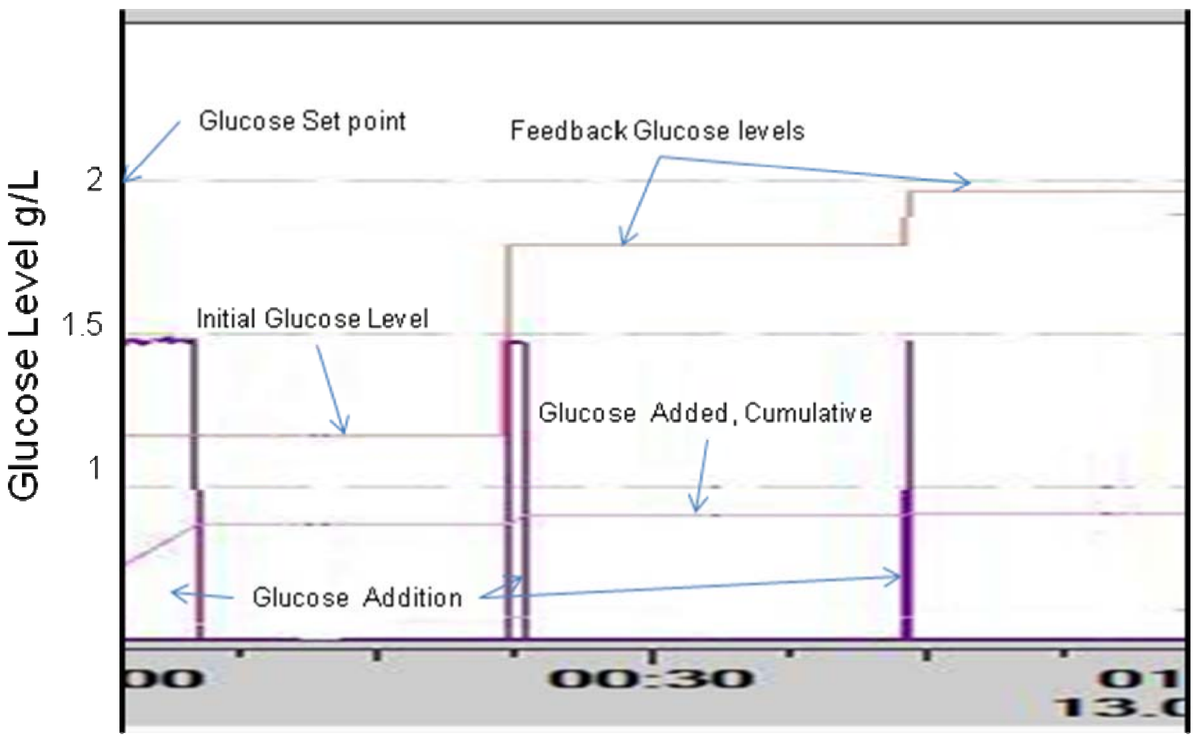

Figure 4: Stepwise Glucose Feedback Control

The Stepwise Feedback control is another feedback control mechanism. The instruction from the Bioreactor Controller is a partial feedback to the set point such that the final set point is accomplished through several feed pump steps converging to the final set point. Note that the change of the programmed feedback control function was implemented by an authorized user during the ongoing experiment; a restart of the controller was not necessary.

In this experiment, as shown in figure 4, the feedback amount of glucose stock was set at 80% of the calculated volume instead of the 100% volume in the batch mode. A total of 4 feeds were required to reach the set point of 2g/L. The data of the steps is shown in Table 1. Note: Some data was omitted in the graph of Figure 4.

Steps	Initial	1	2	3
Glucose Level (g/L)	1.017	1.766	1.970	1.992
Glucose Stock Feedback (mL)	2.729	0.646	0.089	0.011

Table 1: Convergent Steps towards Glucose Set Point

C) Steady Glucose Level Feedback

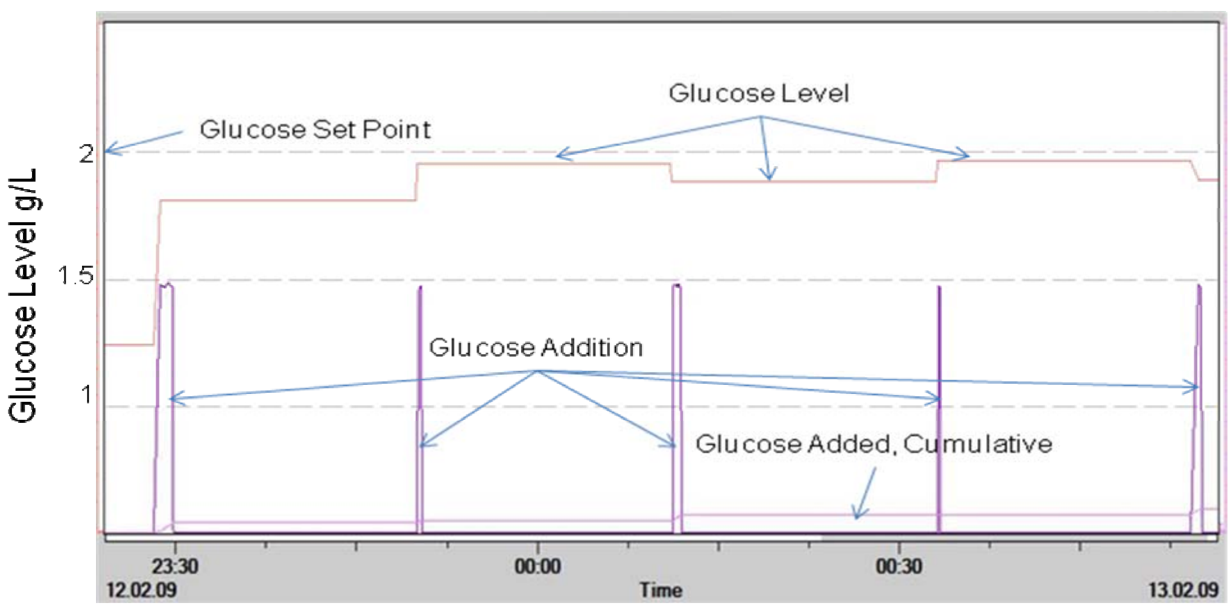

Figure 5: Steady Glucose Level Feedback Control

A more challenging task for a feedback control mechanism is to maintain a steady and optimal glucose (or any other analyte) level throughout a cell culture run while glucose is continuously consumed by the cells metabolism.

In this type of setup the feedback mechanism has to replenish the nutrient consumption (or dilution of metabolites) continuously compared to the required 'bolus injection' as described in the previous batch set up. To accomplish this, the glucose concentration of the medium in the bioreactor needs to be constantly monitored in real time or near real time, in order to activate to feedback loop continuously. The integrated feedback control system described here is ideal for the task.

Reactor 2 was setup to simulate glucose consumption in a live cell culture. This was done by using spare DASGIP system pumps to remove medium from the bioreactor while simultaneously feeding water at an identical rate in order to continuously dilute glucose concentration. This will cause the feedback control system to respond to return the glucose to the set point. Each cycle of the bioreactor/ARS-M/analyzer system took slightly less than 10 minutes. Therefore, with two bioreactors to be sampled in total, each bioreactor was scheduled to be sampled every 20 minutes. The glucose level in bioreactor 2 was maintained at a steady level very close to the set point of 2 g/L after the feedback loop converged at the second step. Please refer to Figure 5 for the feedback process and Table 2 for the experimental data.

Note that this steady feedback loop control was performed while bioreactor 1 batch mode operation was still in progress. This demonstrates the ability of the integrated system to manage several different tasks simultaneously.

Steps	1	2	3	4	5	6
Glucose Level (g/L)	1.781	1.961	1.876	1.969	1.881	1.970
Glucose Stock Feedback (mL)	0.772	0.145	0.428	0.11	0.421	--

Table 2: Steady Glucose Level Feedback

IV. Conclusion

The data is unequivocal that this integrated system provides an Automated Real Time Reactor Feedback Control capability for mammalian cell culture bioreactors. Thus, it would serve as a powerful tool for bioprocess development optimization and eventually large scale manufacturing of medically important proteins.

We thank our collaborators at Groton and YSI for their efforts and cooperation.