Automated Glucose Control for a Cell Culture Process

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Abstract

Manual bioreactor sampling and feeding can be a costly endeavor, both in terms of labor costs and the increased risk for contamination each time the sterile boundary is penetrated. Using the BioFlo® 320 bioprocess control station coupled with BioCommand® SCADA software from Eppendorf, many portions of a cell culture process can be automated, however, the need for human intervention for sample acquisition and analysis remains.
To address this issue, we aim to integrate the BioFlo 320 with the Seg-Flow® 4800 autosampler from Flownamics®, Inc. and the Cedex® Bio HT metabolite analyzer from Roche Diagnostics, GmbH. Together with the automation capabilities of BioCommand, we were able to completely automate glucose control in our feasibility studies. The design of the automation program is fully customizable, allowing the user to implement their own control strategy with custom nutrient target concentration, custom sampling frequencies, etc. Most importantly, the sterile envelope is not penetrated for each sample and feed since the sample is acquired and delivered to the Cedex Bio HT by the Seg-Flow 4800 without human intervention. Following sample analysis, the data is transmitted to BioCommand where the feed program triggers a pump to deliver the appropriate dose of glucose to the vessel through a pre-connected liquid addition port. Using this strategy, glucose and other nutrients can be maintained at a more stable concentration during critical portions of a cell culture process which may have an impact on cell and product yields.

Scope

The scope of this process was to provide a solution for automated glucose monitoring and feeding during a bioprocess run. It is a collaboration between Roche Diagnostics Corporation, Flownamics, Inc., and Eppendorf, Inc.

Material:

Roche
> Cedex Bio HT Analyzer, equipped with reagents to measure glucose

Flownamics
> Seg-Flow 4800, SegMod, and dip tube assembly

Eppendorf
> BioCommand Batch Control Software; BioFlo 320 bioprocess control station and vessel. In the presented study we used a beaker with a stir bar as “mock” bioreactor.

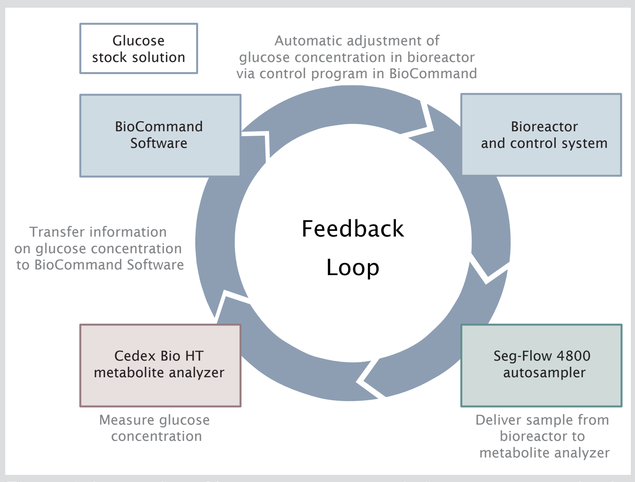
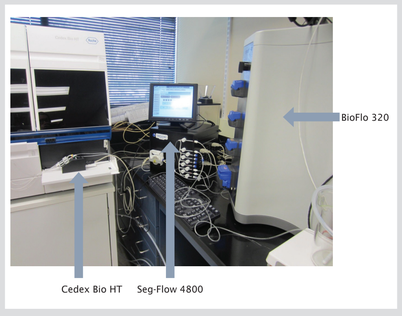


Figure 1: Integration of instruments to control glucose concentration in a feedback loop

Hardware setup



The bioreactor, autosampler, and cell analyzer were connected and the experiment initiated.
The sample and purge volume was 1.25 mL. We used continuous sampling. Each cycle takes about 45 min to complete.

Figure 2: A snapshot of the setup

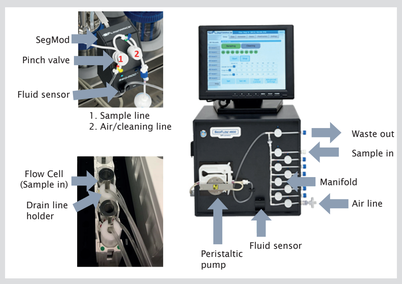


Figure 3: Critical components. **Top left:** The SegMod which uses pinch valves (1, 2) to control flow of air, purge volume, and sample. Also it includes a flow sensor to detect liquid. **Bottom left:** The Flow Cell. Stainless steel tube with a drain on the bottom and inlet port on the upper side wall. The Seg-Flow delivers sample to the upper side wall port which is accessed by the Cedex Bio HT autosampler. Following sampling, the Seg-Flow removes leftover sample using the bottom port and diverts it to waste. **Right:** The Seg-Flow 4800. The manifold and pump are located on this central unit

Software communication

Once the hardware is connected properly and functions, the software communication can be established.

> **Cedex Bio HT Analyzer and Seg-Flow 4800 communication:** Flownamics has established a method of communication between the two instruments by which the Seg-Flow can order tests on the Bio HT automatically and using the Flownamics OPC server, can transfer that data to a bioprocess scada software.

> **Seg-Flow 4800 and BioCommand Batch Control SCADA software communication:**
Using the Flownamics OPC server, certain control loops are read/write-enabled and tags are available to be brought into BioCommand for programming, scripting or track and trending purposes. Available OPC tests:
a) The Bio HT has 43 possible tests and each test has four tags associated (time of the sample, value of the data, unit of the data, and flags for the analysis (errors, warnings, etc.))
b) Start/stop tag. There are four tags per vessel that can be used to start/stop a vessel on the Seg-Flow. It is recommended by Flownamics to use the *Continuous NCommand* tag.
c) Sample frequency tag. The sample frequency (in minutes) on the Seg-Flow can be changed with this tag. In our experiments we did not use the ability to change sample frequency.

A BioCommand recipe was established using the available OPC tags from the Flownamics OPC server. The programming feature was utilized to use the loop data to trigger a glucose feed.

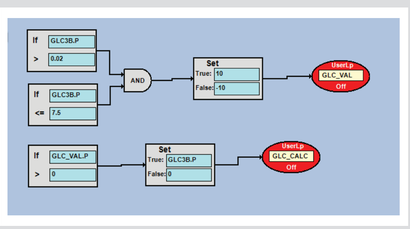


Figure 4: Step 1, validation: This portion of the program determines if the incoming data is within the accuracy range provided by Roche for the glucose test (0.02 g/L–7.5 g/L). Only then will feeding occur.

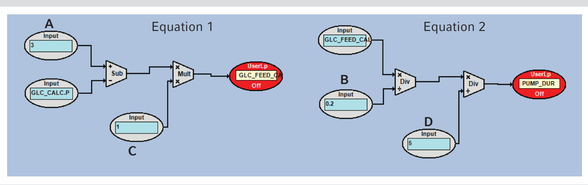


Figure 5: Step 2: Calculates the amount of glucose to feed and converts that value into a time that the glucose feed pump needs to run for (min). The following parameters are customizable:
A. Glucose target concentration in the bioreactor: 3 g/L
B. Glucose concentration of the feed solution: 200 g/L
C. Bioreactor current working volume: 1 L
D. Variable-speed pump setpoint: 5 mL/min

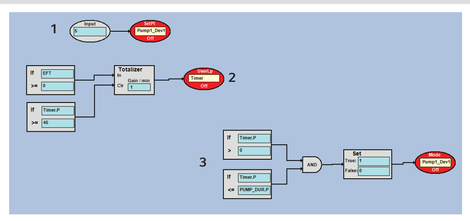


Figure 6: Step 3: Sets the feeding frequency to match the sample frequency and to set the pump to turn on at the right time.
1. Program line to ensure that pump setpoint remained at 5 mL/min. If using a pump that expresses the setpoint in Output %, this parameter can be customized to accommodate.
2. Elapsed fermentation time was used to start a timer that counts to 45 with gain of 1 per min and then resets to zero. Since our sample frequency was set to 45 min, the timer is set to count up to 45. If a different sample frequency is used, this parameter can be customized.
3. Timer was used in combination with IF, THEN logic as a trigger to turn the pump on for the number of minutes calculated in PUMP_DUR loop.

Results

To test the setup, we ran an experiment with a „mock“ bioreactor (2 L beaker with stir bar). To simulate a glucose demand, a part of the volume was replaced with PBS. To validate that the correct amount of glucose was being delivered, an independent analyzer (Roche Cedex Bio) was used to test the glucose concentration at various random time points.

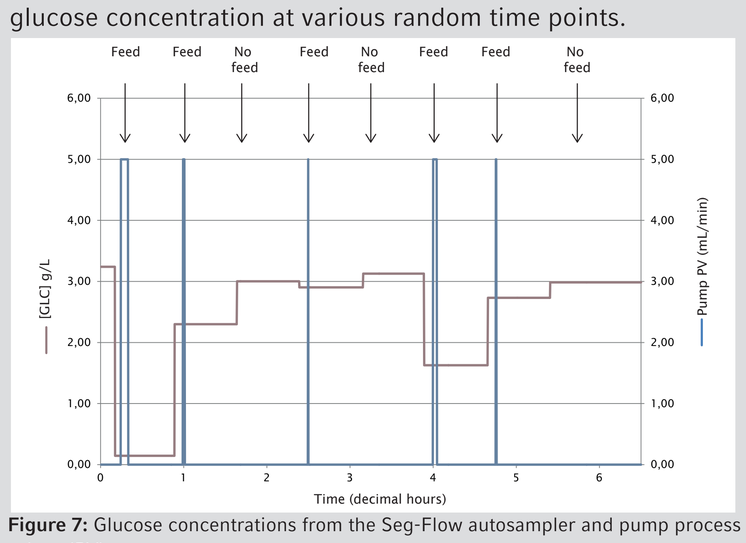


Figure 7: Glucose concentrations from the Seg-Flow autosampler and pump process values (PV).

Table 1: Correlation between Cedex Bio HT data (samples autosampled from the bioreactor using the Seg-Flow 4800) and the data from a manual sample analyzed with the Cedex Bio. The table also notes when the pump was automatically turned on to feed, and what interventions were performed to simulate a glucose demand.

Time (min)	[GLC] (g/L) Cedex Bio HT	[GLC] (g/L) Cedex Bio	Pump action	Note
0	3.24	3.1	N/A	Replace volume with 1x PBS
45	0.14		Feed	
90	2.3	2.55	Feed	
135	3		N/A	
180	2.9	2.89	Feed	
225	3.13	2.89	N/A	
270	1.63	1.64	Feed	Replace 1/2 volume with 1x PBS
315	2.73		Feed	
360	2.98	3.13	N/A	

Conclusion

> A running bioprocess can be automatically maintained at a chosen glucose target concentration using the described setup.

> In this experiment using a mock bioreactor, no cells or particulates were present. Therefore we used the flow sensors and a standard open dip tube for sampling. If a live cell run is performed, the FISP® probe is necessary to remove cells and debris to allow the Seg-Flow 4800 to function properly.

> Less human intervention and calculation reduces the contamination risk, reduces the error rate in feeding, and allows for a higher sampling frequency.

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