Integrated reactor feeding system – amino acid feedback control via online HPLC for productivity gains

Application Note

Biopharmaceuticals

Author

George Barringer, PhD

David Lau, PhD

Groton Biosystems

Boxborough, MA

USA

Abstract

In this Application Note we describe a fully integrated bioreactor system that automates all processes including reactor feeding. The automatic reactor sampling system serves as an enabling control element that links the bioreactor and its reactor control system to at-line and online assay instrumentation. The system has been successfully demonstrated to permit fully automatic analysis and reactor feeding through an entire fermentation process. This system has also demonstrated more than a 30% improvement in product yield.

Agilent Technologies

Introduction

This study demonstrates the efficiency and viability of a fully automatic reactor feeding system to regulate essential amino acid concentrations in a microbial fermentation. These studies were conducted using an online amino acid HPLC system integrated with an ARS-M Reactor sampling system, and a programmable reactor control system controlling amino acid feedstock pumps.

Baseline studies were conducted to determine the amino acids that were consumed by the fermentation process and the rates of consumption. Subsequent studies were conducted in which those amino acids were fed to maintain a desired concentration for the entire course of the fermentation. Green Fluorescent Protein ("GFP") was used as a marker for specific productivity measurement. Initial results indicate that productivity is positively correlated to maintaining appropriate levels of essential amino acids. Future studies are in progress to optimize the media and feedstocks.

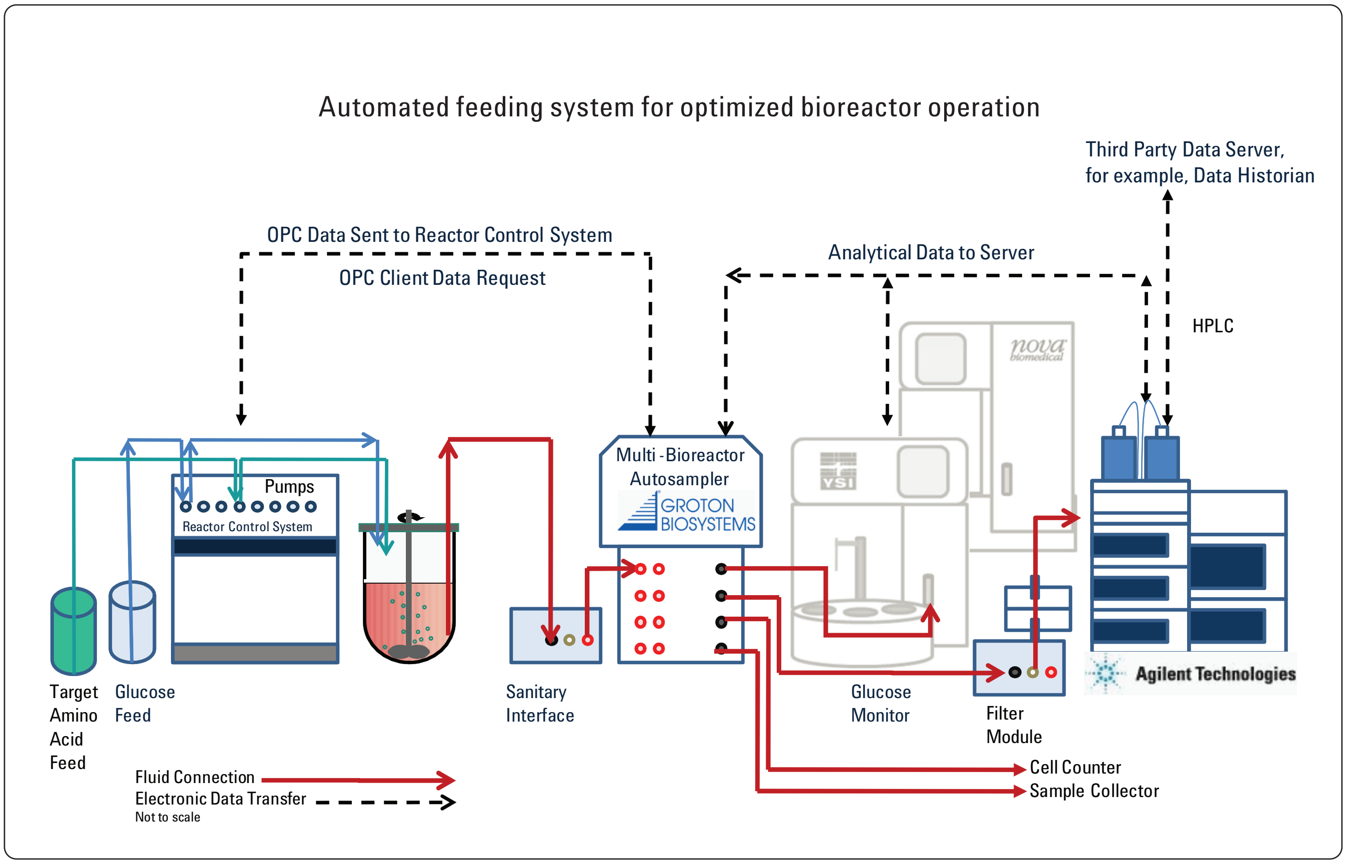

Figure 1
Integrated, automated bioreactor system.

Experimental Method

Conditions	
Sample:	Microbial (*E.Coli*) – rGFP-BL21 – casamino acid media
Sampling system:	Groton Biosystems ARS-M440
Reactor:	DASGIP 1.5 L Glass Bioreactor
Reactor interface:	Groton Biosystems *In-situ* Probe and iRIM
Assay interface	Groton Biosystems Agilent HPLC Interface with OPC Kit
Output assay:	Agilent 1200 Series LC system – Agilent AAA Amino Acid Method Groton HPLC Data Wizard Groton Biosystems Sample Collector – GFP and Negative Control Samples
Process:	1 Day process

Fermentations were performed using stock inoculation cultures and stock media. The cycle ran for 24 hours. Samples were extracted periodically by the ARS-M through the course of the cycle and submitted automatically to the HPLC for amino acid analysis by the standard method. Data was reduced to typical report form by the HPLC operating system software. Selected chromatogram data sets were identified by the user in a prerun setup process, then extracted automatically from the chromatogram report on each subsequent chromatogram assay by the Groton HPLC Data Wizard for retransmission to the Reactor Control System. The Reactor Control System pump control algorithms reduced each selected chromatogram concentration data point to a process value, compared that value to a desired set point value, then calculated a correct feed pump duty cycle for the next forward interval. This process iterated to the end of the fermentation cycle.

Results

Amino Acid Baseline – BL21 *E.Coli*
A baseline experiment was performed using stock culture and stock media. Feeding was not performed in order to map the consumption rates of amino acids to determine potential single essential amino acid feedstocks. Raw chromatograms from AAA assay are shown (Figure 2) at selected times in the fermentation cycle.

Note the consumption of serine (Figure 3). Other amino acids also exhibited consumption or expression during the fermentation. Serine was selected for the feeding evaluation (Figure 4).

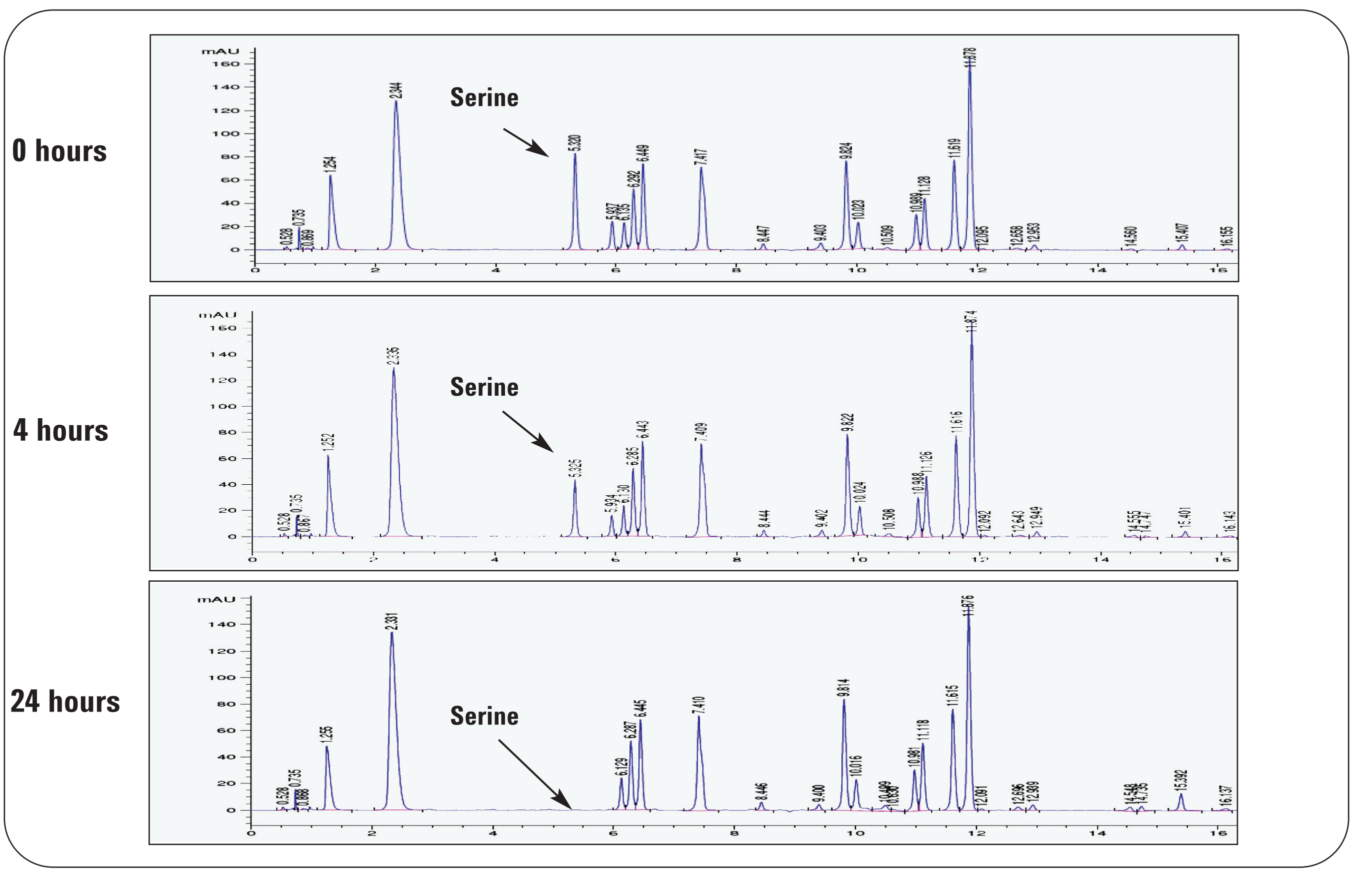

Figure 2
Raw amino acid chromatograms – no feedback control.

Serine feedback control – Bioreactor profile with OPC, rBL21

Feedback control was enabled for the reactor via the Groton ARS-M Agilent HPLC with an OPC Interface Kit connected to the reactor control system. This kit acquires automatically selected chromatogram data from each chromatogram, stores the data in an OPC server, and retransmits the data on request from any OPC-enabled client. In this case, this is the Reactor Control System enabled to programmatically control duty cycles for up to six feed pumps per reactor. The selected amino acid for assay and feeding for this demonstration was serine (Figure 5).

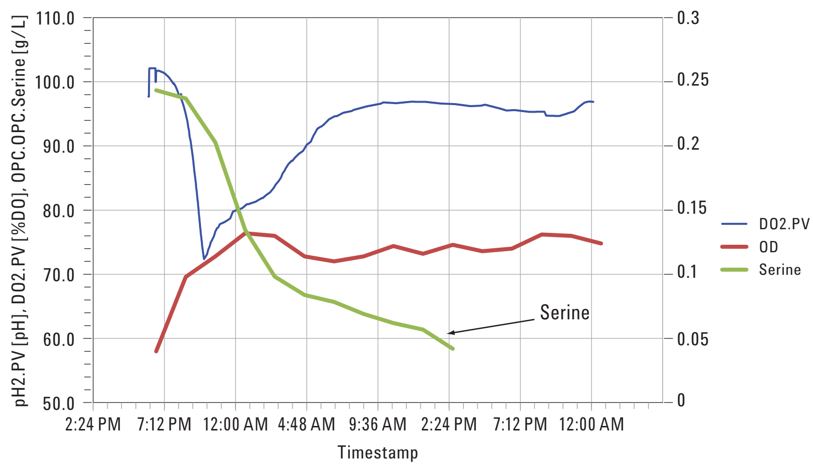

Figure 3
No Feedback Control, rBL21; Selected process value plots from reactor control system dO2 – Light Blue; OD – Red; Serine – Green. Derived from chromatogram data.

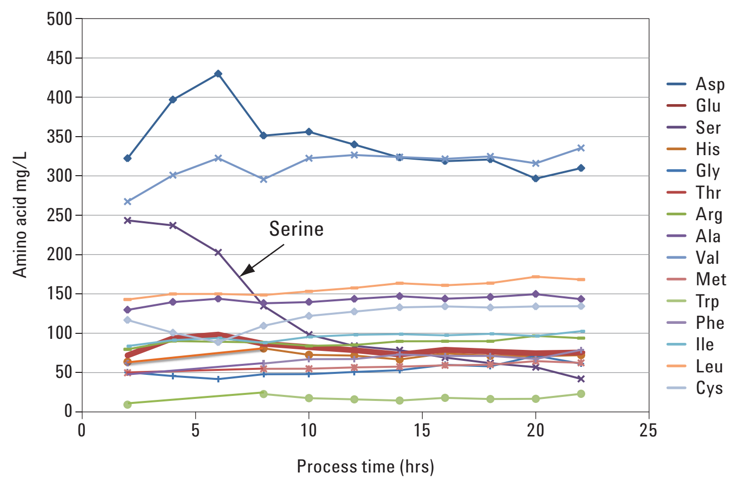

Figure 4
No Feedback Control; Amino acid profile, rBL21; All amino acids above limit of detection Casamino acid media. Derived from chromatogram data.

In this experiment manual samples were collected periodically (dark blue trace in Figure 5) by extraction through a dip tube using a syringe. The samples were analyzed by standard techniques with the same HPLC and method used automatically by the ARS-M. These samples were filtered according to the same criteria used by the ARS-M automatic sampling system; cell free filtered to 0.22 µm and stored at 4 °C until assayed. The automatically-collected and processed serine data are shown in light green (Figure 5). Each horizontal segment of the trace represents the process value acquired by HPLC AAA assay as transmitted to the reactor control system. The system was programmed to acquire samples and perform the AAA assay periodically through the course of the entire fermentation cycle. Note the correlation between the automatic and the manual ("standard") assay values (Figure 5).

The red trace at the bottom of the screen represents the duty cycle trace for the serine feed pump. The feed pump was set to a constant flow rate. The reactor control system proportionally turned the pump on and off to achieve an overall serine feed in units of mg/min of "on" time. Note that the duty cycle is inversely proportional to OPC serine process value; long when concentration is low, short when concentration is high.

Figure 6 shows the time course of serine concentration through the entire process cycle.

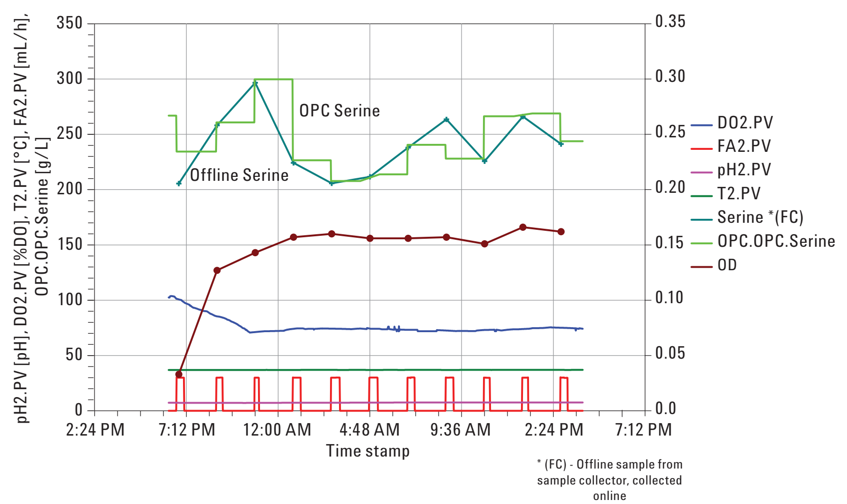

Figure 5

Serine Feedback Control – Bioreactor Profile with OPC, rBL21; reactor control system process value plots for selected parameters

OPC Serine Process Value – Light Green; Manual Serine – Dark Blue; OD – Red; dO2 – Light Blue Temperature – Green; Serine Feed Pump Duty Cycle Modulated - Red

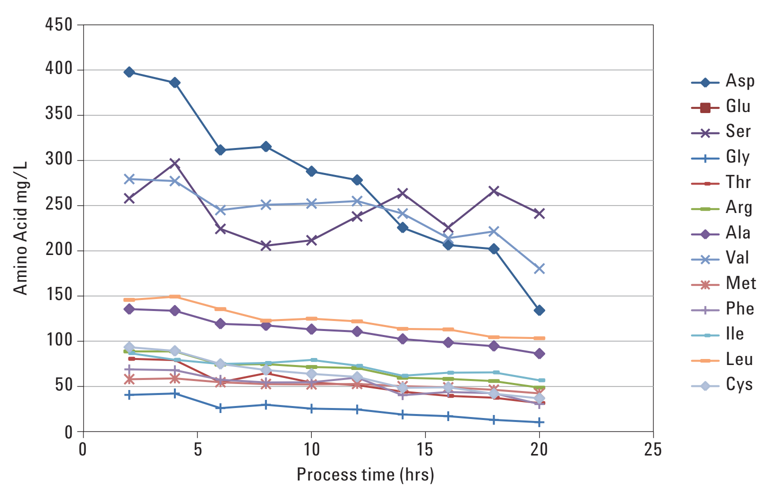

Figure 6

All amino acids showing serine controlled at original and selected concentration of 250 mg/mL.

rGFP-BL21 Serine Feedback Productivity

An initial study was performed to demonstrate the correlation of structured amino acid feeding to productivity of the fermentation process. The selected *E.coli* strain expresses the Green Fluorescent Protein (GFP) that was used as a marker for specific productivity in this study.

In this study, a control experiment was performed as described above to measure GFP titre under control conditions (no feeding). This is shown in Figure 7. In the controlled feeding study GFP (dark blue) is shown to yield 33% more product with continual feeding of one amino acid, serine, but otherwise identical culture conditions.

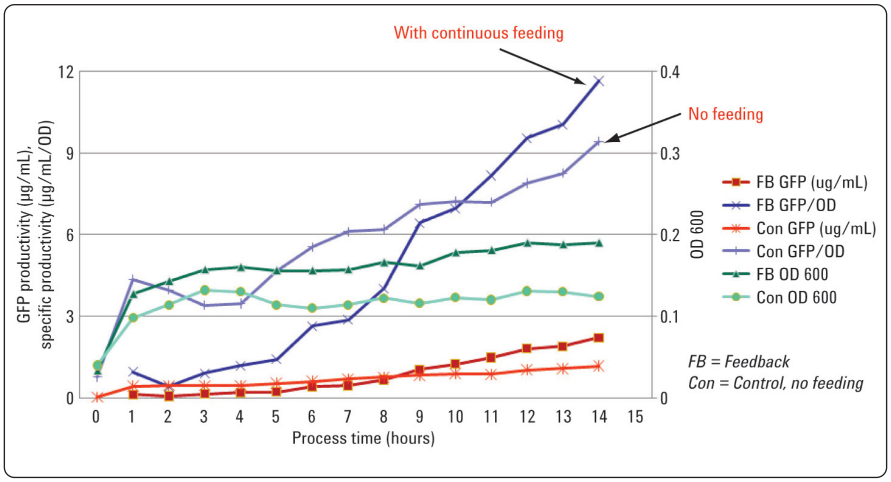

Figure 7
Controlled feeding study measuring GFP titre.

Conclusion

Strategic feeding of bioreactors under controlled conditions using real time feedback control and intelligent algorithms is shown to provide an improvement in yield of desired products. The yield of GFP, the marker protein tracked in this current study, increased by 33% through intelligent feeding of one amino acid, serine, shown to be essential for this *E.coli* strain.

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