

Automated Sanitary Sampling Optimizing Bioreactor Product Quality and Yield

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High product quality and yield are the goals in production of recombinant therapeutic proteins, polysaccharides, and other pharmaceutical compounds (and their precursors). Appropriate monitoring of bioreactor cell growth, product expression, and feedback can dramatically increase quality and yield, providing significant economic benefit to biotechnology manufacturers. For a perspective, the US Food and Drug Administration (FDA) recently indicated that improved process information and control have been shown to cut cycle times by as much as 50% in some applications (1).

Although process monitoring has been routinely used in many industries (e.g., the semiconductor, petroleum, chemical, and smelting industries) for a long time, it has been more recently adopted by the biotechnology industry. A recent *Wall Street Journal* report indicates that "the pharmaceutical industry has a little secret: Even as it invents futuristic new drugs, its manufacturing techniques lag far behind those of potato-chip and laundry-soap makers" (2). In recent years, the FDA has addressed the issue of process control in

pharmaceutical manufacturing and strongly urged the industry to adopt advanced process analytical instrumentation similar to that used in other industries. The agency recently charged its Process Analytical Technology (PAT) subcommittee with helping the biopharmaceutical industry develop process instrumentation for bioreactor monitoring and control.

Drawbacks to Manual Sampling for Fermentation or Cell Culture: In spite of the benefits of process monitoring, many facilities do not routinely sample their bioreactors. There are several reasons for this.

Manual sampling can be tedious and expensive, especially if it is required 24 hours a day, seven days a week. The typical reaction cycle for a mammalian cell culture is about 14 days, and the cell count (and protein expressed) doubles every 24 hours. Similarly, a typical microbial fermentation cycle is 16–24 hours, and the cell count (and protein expressed) can double in less than an hour. So a user could perform an analysis every hour to monitor either process. Additionally, manual sampling can be subject to experimental, custody, and labeling errors, and the results can depend on the skill of individual operators. The sampling process itself can increase the risk of bioreactor contamination.

The two discrete steps in sampling a bioreaction are acquiring a sample and analyzing it for the compound(s) of interest. Here I describe a new automated reactor sampling (ARS) system that automates sanitary sampling from a bioreactor (see the "System Configurations" box). It can interface with a range of analytical instruments for on-line analysis or a fraction collector for off-line analysis (Photo 1).



Photo 1 (WWW.GROTONBIOSYSTEMS.COM)

CRITICAL STEPS IN AUTOMATED SAMPLING

The three steps in automated bioprocessor sampling with this system are withdrawing a sample from a reactor, transferring it to the ARS, then transferring it to an analytical instrument (Figure 1).

Withdrawing a Sample: Operators can set the sample volumes and flow rates. The automated system is programmed to withdraw a sample from a reactor or to do so on demand from an operator. Sample flows through a remove valve interface (RVI) that isolates the reactor from the sample handling system to ensure integrity of both the sample and the reactor contents. Our system can be configured with a variety of probes depending on the application: cell-free sample probes such as for membrane filtration (in situ) and in-line disposable membrane filters, as well as cell-containing sample probes (dip tube and specially designed CIP/SIP probes).

Transferring Samples: Samples are transferred from the RVI into the ARS using a series of isolation valves so as not to compromise the purity of the bioreactor and sample.

Transferring Samples to an Analytical Instrument: Once a sample

PRODUCT FOCUS: ALL BIOLOGICALS

PROCESS FOCUS: PRODUCTION
(CELL-GROWTH MONITORING)

WHO SHOULD READ: PRODUCTION
ENGINEERS, CELL CULTURE ENGINEERS

KEYWORDS: BIOREACTORS, CELL
CULTURE, FERMENTATION, FRACTION
COLLECTORS, BIOCHEMISTRY ANALYZER

LEVEL: INTRODUCTION

has been drawn into the ARS System (and is isolated from the reactor), it is then transferred to an appropriate analytical system for on-line analysis or to a cooled fraction collector for off-line analysis. This transfer is performed using a series of valves and a syringe pump similar to that used in the previous transfer to the system.

POSTSAMPLING ANALYSIS

The Groton ARS system is adaptable to a number of analytical interfaces and can be directly connected to an instrument for on-line analysis. A sample is delivered to the inlet of the instrument, and the instrument is programmed to perform the desired analysis. As an alternative, the ARS can deliver the samples to an automated fraction collector for off-line analysis or separation by HPLC.

On-Line Analysis: A typical example of on-line analysis is an interface of the ARS with the YSI Model 2700 (www.ysi.com). This is an enzyme/substrate-based electrode system that can measure glucose and lactate simultaneously in 1.5–4.0 minutes, depending on the analyte. Table 1 shows the analytical range that can be monitored for dextrose. Data from the Model 2700 are transmitted to the ARS control system through RS-232 or RS-485 serial ports. The YSI system is locally programmed and operates from ARS software: The ARS sends a signal to it for calibration, sample analysis, and so on.

Off-line Analysis: When off-line analysis is used to determine the concentration of a compound of interest, samples are diverted to a fraction collector. We use a self-contained, refrigerated unit that preserves sample integrity, eliminating the need to perform collection in a cold room.

USER CONTROL AND AUTOMATION

Our system is controlled through a user-friendly interface that provides for spot sampling and time-based programmed sampling (periodically or at user-specified times). A user can generate a sampling method that indicates critical parameters such as the volume of fluid to be withdrawn, priming pumps flow rate, and the number of times the system should perform various sampling actions (such as sample draw and system cleaning). A user can generate an analysis method, such as

to advance the fraction collector or issue a command for the analyzer to perform a self-calibration before analysis. An established method can be saved and recalled as required.

The main screen (Figure 2) presents an overview of the various system operations, with the present operation highlighted for ready reference. When the START command is given, the desired method will perform the appropriate steps automatically. All operations of the various subsystems (opening/closing valves, moving fluid) are controlled by a script that is specifically prepared to optimize the overall process.

The system is designed to meet 21 CFR Part 11 requirements. During operation, the execution of each step is recorded in a log for permanent reference. System validation packages have been developed to help users meet CGMP standards. The instrument can be operated locally or with feedback through a peer-to-peer network with other components of a facility.

SANITATION ISSUES

The ARS system incorporates automated CIP protocols in its methods. These are specifically designed to meet end-user requirements, and the system can be cleaned with a broad range of reagents, including sodium hydroxide or sodium hypochlorite. Also, operators can

perform additional cleaning protocols as desired. Sterilization (SIP) protocols can be performed with reagents such as ethanol, formalin, or sodium hypochlorite. It also is possible to steam sterilize connections from the reactor to the remote valve interface.

PERFORMANCE STUDIES

Maintaining Sanitary Conditions: A number of experiments demonstrate that automated sampling does not contaminate bioreactors. In one experiment, the ARS and RVI were sterilized with 70% ethanol before connection to a bioreactor containing animal cells. We monitored oxygen levels over a complete six-day period in which 20 samples were withdrawn. During the first four days, the dissolved oxygen (DO) level remained at the set point of 50%, and afterward the oxygen level increased to almost 99% due to the anticipated death of the animal cells. If the ARS operation had contaminated the reactor, we would have expected the oxygen level to be very close to zero because bacteria use oxygen rapidly.

To provide further evidence that sampling did not contaminate the reaction mixture, a 400× magnification of the animal cells by a CCD microscope showed

Figure 1: Steps in sanitary sampling

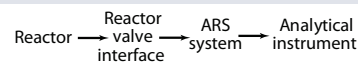


Table 1: Comparison of manual and automated sampling

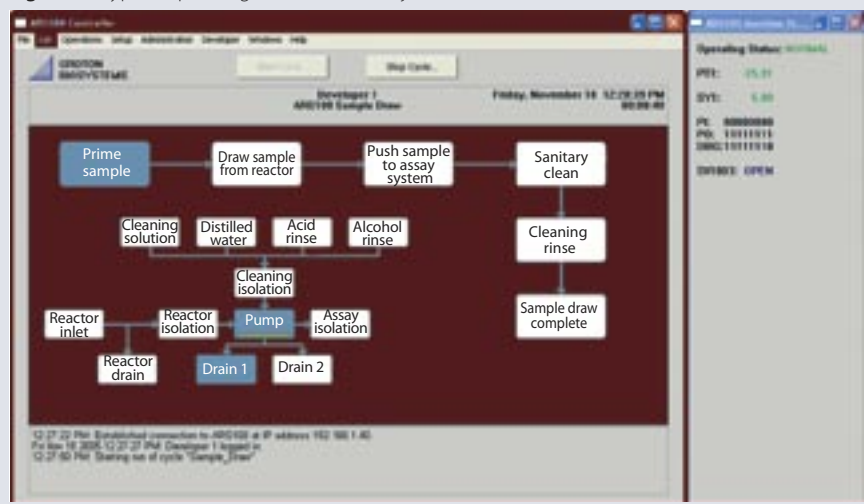
	Low Dextrose ~0.225 g/L		Mid Dextrose ~9.0 g/L		High Dextrose ~25.0 g/L	
	YSI	ARS-YSI	YSI	ARS-YSI	YSI	ARS-YSI
Average (N = 6)	0.222	0.220	8.83	8.74	24.7	24.4
Standard Deviation	0.0009	0.0018	0.0226	0.039	0.31	0.44
CV	0.40%	0.81%	0.26%	0.45%	1.26	1.81

SYSTEM CONFIGURATIONS

The Groton ARS system is scalable from development to production systems and is designed to be compatible with bioreactors ranging in volume from 1 to 20,000 L. Each system is configured to a specific application. Wetted surfaces meet chemical compatibility requirements of the sample (typically PTFE, PEEK, stainless steel [type 316], Chemraz, or EPDM).

For development systems, diaphragm valves are electronically activated; for production systems, standard, pneumatically activated biotechnology diaphragm valves are used. Multiplexed versions can be configured to sample from one to four bioreactors, and samples from each bioreactor can be delivered discretely to four analytical modules.

Figure 2: Typical operating screen (colors adjusted)



period of time and through a series of repetitive sampling procedures. In addition, data demonstrate that samples withdrawn from a bioreactor are equivalent to those manually acquired in terms of sample concentration and properties of the cells under investigation. Clearly, the ARS system can significantly reduce the time and cost of routine sampling from a bioreactor, making it an extremely valuable tool for providing rapid and timely feedback and thereby meeting PAT requirements.

ACKNOWLEDGMENTS

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FOR FURTHER READING

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Table 2: Comparison of cell measurements between manual sampling and ARS

	Manual	ARS	Variation (%)
Viable Cell Density	17.98	18.15	1
Total Cell Density	44.40	45.06	1
Viability	40.50	40.30	0
Total Cell Count	3887	3719	4
Average Compactness	1.10	1.12	2
Average Diameter	12.94	12.91	0
Standard Deviation	2.58	4.05	
Off-Line pH	6.86	6.86	0
Average Variation			1

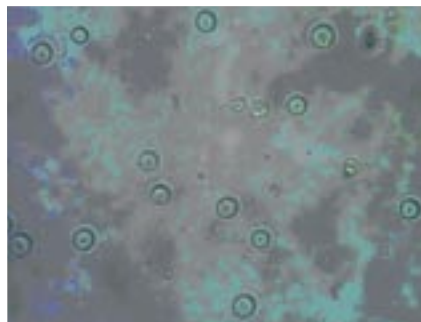


Photo 2: Photograph of animal cells from bioreactor after 20 sample withdrawal cycles in six days. The cell viability indicates that no contamination from sampling had occurred. WWW.GROTONBIOSYSTEMS.COM

volumes to the instruments were 4.0 mL (both at a flow rate of 5.0 mL/min).

Maintaining Cell Viability: An important issue in bioreactor sampling is ensuring that a sampling process does not lyse or damage the sample cells. To test this, we withdrew animal cells from a 1.7-L bioreactor both manually and with the ARS. An Innovatis Cedex cell analyzer (www.innovatis.com) was used to measure a number of cell parameters. The results, presented in Table 2, clearly indicate that sampling a bioreactor using the ARS does not affect cell viability.

COMPLYING WITH PAT

The ARS system is designed to provide automated sanitary sampling in a broad range of bioprocess applications, including vaccine production, drug (and drug metabolite) production, and other areas requiring routine monitoring of a bioreactor or process stream. Our data clearly indicate that sanitary conditions in the bioreactor are maintained during the ARS sampling process over an extended

no bacterial rodlike cells. Photo 2 clearly indicates the integrity of the cells and an absence of bacterial contamination.

Accuracy and Precision of the Sampling Process: A series of parallel tests demonstrated that the ARS did not affect the accuracy of the analytical measurement. Samples were manually withdrawn, using the ARS, from a bottle containing a dextrose solution. They were analyzed by the YSI 2700. Three series of experiments were performed, and the data in Table 1 clearly indicate that the ARS sampling is equivalent to manual sampling. Each sample cycle included a cleaning cycle with 0.1 N sodium hydroxide, followed by neutralization with 0.1 N acetic acid, a water rinse, and then a sterilization cycle with 70% ethanol. Input volumes from the reactor were 7.0 mL, and output