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Intelligent Control of Chinese Hamster Ovary (CHO) Cell Culture Using the BioFlo® 320 Bioprocess Control Station

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Abstract

The recently released BioFlo 320 bioprocess control station offers some of the most intelligent cell culture control mechanisms on the market today. The innovative intelligent software automatically recognizes different type of sensors, from conventional analog sensors to proprietary sensors equipped with Intelligent Sensor Management (ISM®) technology by Mettler-Toledo®; from traditional polarographic sensors to advanced digital and optical sensors. The BioFlo 320 also offers flexible connections to either traditional glass vessels or various BioBLU® single-use vessels without relying on external adaptors. Combined with the seamless integration of biomass sensors from FOGALE nanotech®, the BioFlo 320 presents an intelligent setup with which to conduct and monitor mammalian cell culture. In this application note, Chinese Hamster Ovary (CHO) batch cell culture runs were conducted to highlight the versatility of this new control station, and as such, various sensors and control strategies were employed. First, the new capability of the control station to automatically detect and integrate sensors with ISM technology was utilized. Sensor health and maintenance was monitored using iSense software (Mettler-Toledo). In addition, the evo 200 (FOGALE nanotech) capacitance-based biomass sensor was also included for in-line growth monitoring. The ease of sensor detection and calibration combined with the elimination of the need for offline cell counting elevates this experiment to "intelligent" cell culture.

Introduction

The BioFlo 320 combines features and benefits from the New Brunswick™ BioFlo/CelliGen® 310 benchtop, autoclavable bioreactor system and the New Brunswick CelliGen BLU bioreactor to create an all-in-one bioprocess system with unique capabilities for intelligent cell culture (Figure 1). The BioFlo 320 can interchangeably control industry-standard autoclavable glass vessels or BioBLU single-use vessels. In addition to increased versatility with respect to vessels, the BioFlo 320 offers the ability to seamlessly connect a wide variety of Mettler-Toledo ISM sensors including dissolved oxygen (DO) and carbon dioxide (DCO_2), pH, and redox. As with previous models, the BioFlo 320 supports 4 – 20 mA input/output connection with a multitude of ancillary devices including auxiliary

Figure 1: The left and right-handed BioFlo 320 bioprocess control stations with magnetic drive glass water-jacketed vessel (left) and BioBLU single-use vessel (right)

pumps, turbidity sensors, capacitance sensors, extra scales, automatic samplers, and biochemical analyzers, which can be recorded and/or controlled within the software.

In this work, the BioFlo 320 was used to control two batch suspension CHO cultures in a 3 L glass water-jacketed vessel. The runs differed in the automatic gassing strategy employed: one run used the 3-Gas algorithm and the other run used the 4-Gas option. In addition, to highlight the ability to integrate many different sensor types, three different DO sensors were used to monitor the DO levels in both cultures: (1) an ISM polarographic DO sensor, (2) an ISM optical DO sensor and (3) an analog polarographic DO sensor. Using the ISM-compatible BioFlo 320 software paired with the Mettler-Toledo iSense software, users can monitor sensor health, lifetime, calibration data, and autoclave/sterilization times, among other parameters. During these runs, both gassing strategies resulted in an average peak density of 9 x 10^6 cells/mL before nutrient depletion occurred.

Materials and Methods

Tables 1 and 2 outline the hardware and consumable reagents used in this study.

Vessel preparation
A 3 L glass water-jacketed vessel with magnetic drive and pitched blade impeller was outfitted with 3 DO sensors (see Table 1), an ISM pH sensor and an evo 200 biomass sensor. All 3 DO sensors were placed directly next to one another at the same height in the vessel. The headplate was also fitted with an exhaust condenser, thermowell, ring sparger (macrosparger), harvest dip tube, sampling dip tube, and 2 liquid addition ports (one for media addition and the other for base addition). The vessel was autoclaved with 2 L of phosphate buffered saline (PBS) and the water jacket half filled with water.

Sensor calibration, monitoring, and troubleshooting
Prior to autoclaving the vessel, an ISM gel-filled pH sensor (see Table 1) was connected to the BioFlo 320 control station where it was automatically detected by the control station software. Calibration was performed according to the operating manual using buffers of pH 7 and pH 4 for "zero" and "span," respectively. Unlike an analog sensor which stores calibration data only in the control software, the calibration data is stored in the ISM pH sensor itself, allowing it to be recalled at any time. In addition, the sensor can be connected to the optional Mettler-Toledo iSense software via the iSense USB adapter. Using this software, a wide range of data is available including the calibration data performed in preparation for this experiment as well as intelligent monitoring of a sensor's remaining shelf life (sensor "health"). Figure 2 illustrates the "ISM Monitor" screen available in the iSense software. The indicators on this and other screens within use green, yellow, or red icons to show sensor status.

Parameter	Configuration	Setpoint
Vessel	3 L glass water-jacketed vessel	3.75 L working volume
Gassing Control	Four Thermal Mass Flow Controllers (TMFCs) with 0.002 – 1 SLPM flow range	Automatic 3-Gas or 4-Gas mix
Sparge	Ring sparger (Macrosparge)	N/A
DO sensors	ISM polarographic (InPro® 6800) ISM optical (InPro 6860i) Analog polarographic (InPro 6820)	50 % (controlled by the ISM polarographic sensor)
Agitation	Magnetic drive	80 rpm
Impeller	Pitched blade impeller	N/A
pH sensor	ISM gel-filled InPro 3253i pH sensor	7.2 (0.05 dead band)
Temperature	N/A	37 °C

Table 1: BioFlo 320 hardware configuration and setpoints

Material	Supplier	Catalog No.
Freestyle™ CHO-S	Life Technologies®	R800-70
Phosphate Buffered Saline (PBS)	Fisher Scientific®	BP399-500
CD-CHO Media	Life Technologies®	10743
L-glutamine	Life Technologies®	25030
Sodium bicarbonate	Fisher Scientific®	S631-3

Table 2: Reagents used in this study

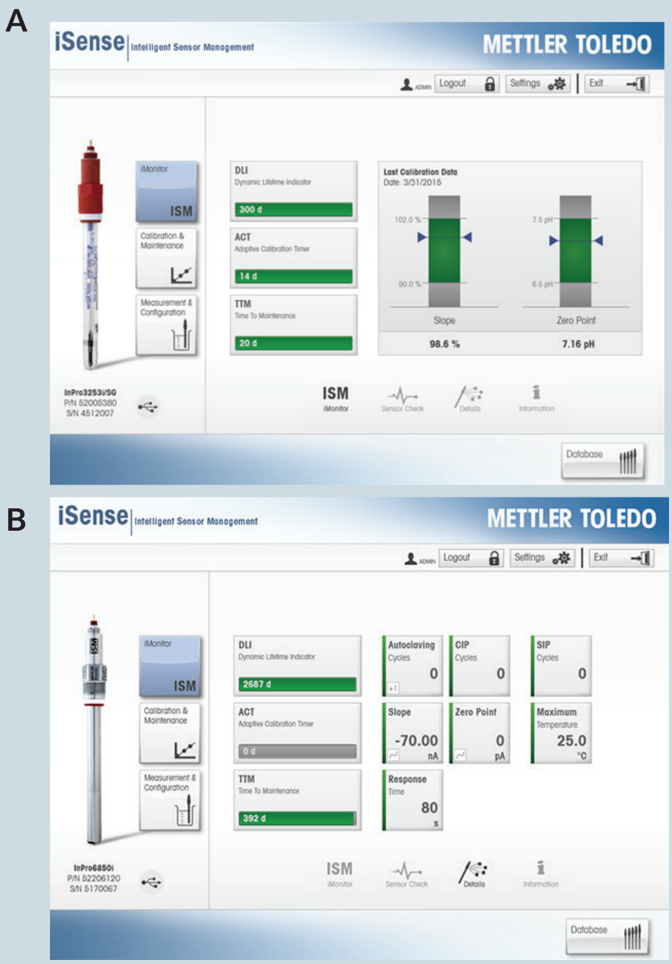

Figure 2: Panels A and B illustrate screenshots from the Mettler-Toledo iSense software which allows the user to monitor sensor "health"; calibration data, configuration, and sterilization cycles can be tracked

Results and Discussion

As seen in Figure 3, both the 3-Gas and 4-Gas automatic DO control algorithms allowed the culture to reach similarly high viable cell densities. The 4-Gas experiment reached its peak cell density (8.89 x 10^6 cells/mL) sooner than the 3-Gas run (9.54 x 10^6 cells/mL). In addition, Figure 4 shows that glucose consumption and lactate and ammonia accumulation were comparable between the two cultures. Consistent with the cell density trend, the 3-Gas culture consumed glucose slightly slower than the 4-Gas culture. When the glucose was exhausted, the cell growth and viability began to drop. Higher peak densities would have been possible if glucose and other necessary nutrients had been supplemented using a fed-batch protocol.

The two gassing control algorithms produced comparably healthy cultures, and showed some notable gas consumption differences. The 4-Gas culture consumed more gas overall, as illustrated in Figure 4D. Since the 3-Gas algorithm does not utilize N_2 for DO control, there is a possibility for the DO to climb above setpoint at the beginning and end of the run when O_2 demand is low. Using 4-Gas control, N_2 is available to keep DO at setpoint, which may be beneficial for some sensitive cell types, and for anaerobic cultures. Whether a culture will be healthier with 3-Gas or 4-Gas automatic gassing control will have to be determined empirically for each cell strain.

The evo 200 capacitance biomass sensor was a valuable in-line measure of cell growth during the runs. Figure 5 shows a comparison between the offline viable cell density measurement and the in-line evo 200 capacitance measurement for one run. After calibrating this sensor for a particular cell line and specific culture process, it can be used in place of sampling the bioreactor which would avoid lost volume and reduce the risk of contamination.

Three DO sensors were incorporated into these experiments. The two ISM sensors were automatically detected by the control station, and including the traditional polarographic sensor, all three were able to accurately track and trend DO levels throughout the run. Figure 6 illustrates an example of the DO sensor trends for the 3-Gas experiment. No significant differences were seen between DO measurement by the three sensors.

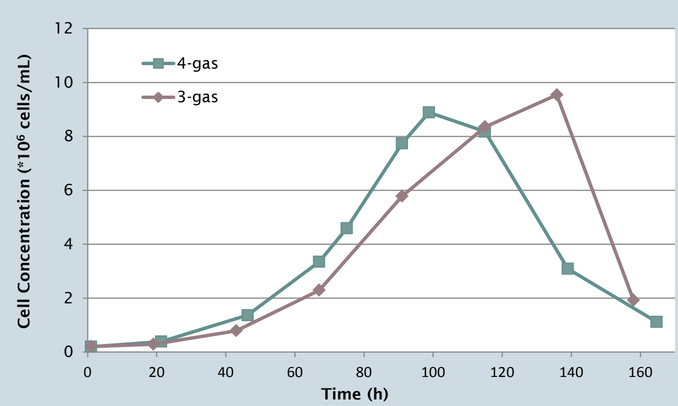

Figure 3: Viable cell densities between the 3-Gas and 4-Gas experiments; although the 3-Gas run peaked a day after the 4-Gas run, peak cell densities were not significantly different

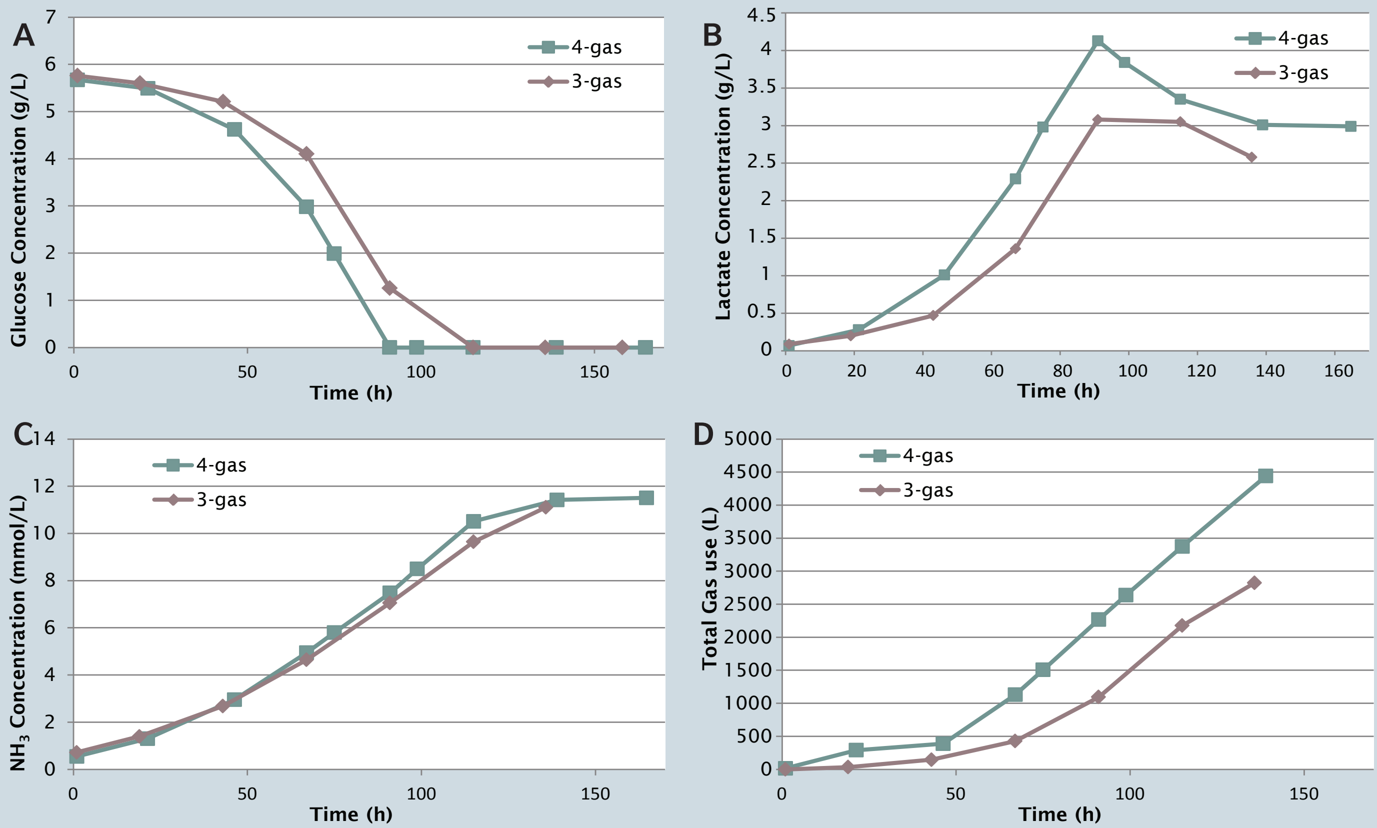

Figure 4: Comparing **(A)** glucose, **(B)** lactate, **(C)** ammonia concentrations, and **(D)** the total gas consumption between 3-Gas and 4-Gas during the bioreactor runs

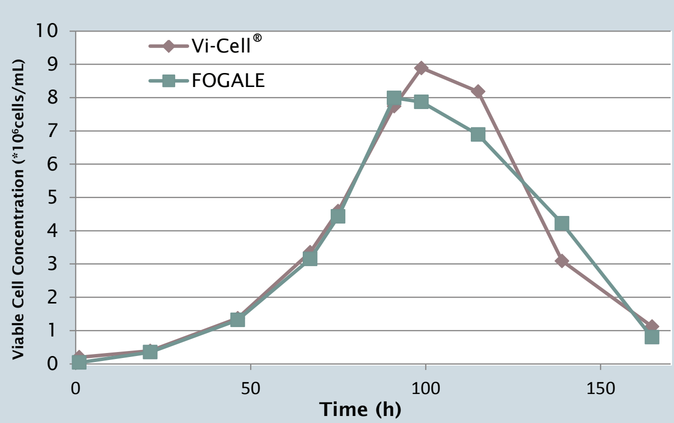

Figure 5: Comparing viable cell concentration between an offline cell count and readings taken from the evo 200 biomass sensor at each sample point

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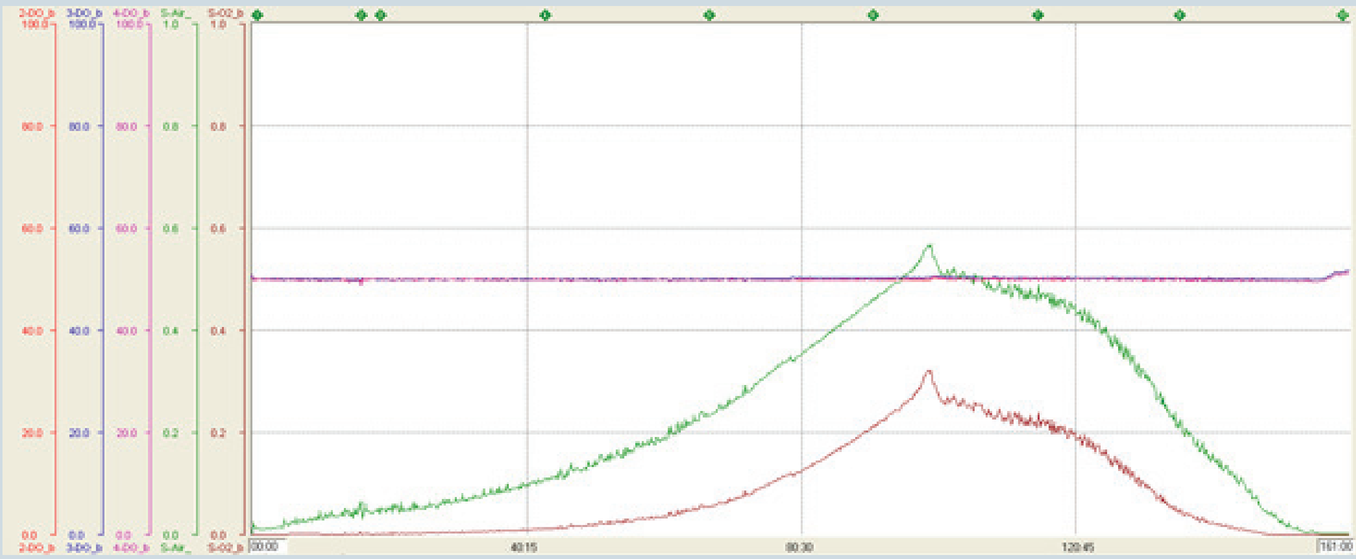

Figure 6: This trend was generated by BioCommand® Batch Control which was used to collect data from all control loops through the runs. In this example, the three DO sensors (red, blue, and pink), sparge air (green) and sparge O_2 (maroon) trends are shown during the 3-Gas run. Note that the three DO trends are superimposed on one another.

Conclusion

With the intelligent upgrades to the BioFlo 320 software and the utilization of intelligent pH/DO sensors, the BioFlo 320 provides advanced process control for CHO cell culture. This method provided similar results using either the 3-Gas or 4-Gas automatic gassing cascades. The setup can be used to meet a host of culture requirements and the upfront knowledge of an ISM sensor's "health" dramatically reduces operational risk due to potential sensor failure during a cell culture run. In these experiments, the ability to customize the configuration by adding an evo 200 biomass sensor and multiple ISM DO sensors elevated these runs to "intelligent" CHO cell culture. With the addition of an in-line bioanalyzer, sampling of the bioreactor could be eliminated to reduce the risk of sampling-associated contamination, making the BioFlo 320 a superior setup for cell culture and an intelligent choice for bioprocess laboratories worldwide.

Ordering information

Description	Order no. International	Order no. North America
BioFlo® 320 Bioprocess Control Station	Please Inquire	Please Inquire
BioFlo® 320, 3 L Vessel Bundle, water-jacketed, magnetic drive	M1379-0311	M1379-0311

Your local distributor: www.eppendorf.com/contact
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