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

Advances in bioprocess control technology

Intelligent control in cell culture process development. By Christiane Schlottbom, Stacey Willard & Ma Sha

In industrial production of proteins and antibodies, Chinese hamster ovary (CHO) cells have been established as the number one mammalian host. New sensor technologies and intelligent bioprocess control stations can enhance process development in the bench scale and hence pave the way for scale up.

Advanced bioprocess development

The recently released Eppendorf BioFlo 320 benchtop bioprocess control station can interchangeably control industry-standard autoclavable glass vessels or BioBLU Single-Use Vessels. In addition to increased versatility with respect to vessels, it 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. The BioFlo 320 supports 4-20 mA input/output connection with a multitude of ancillary devices including auxiliary pumps, turbidity sensors, capacitance sensors, extra scales, automatic samplers, and biochemical analysers, which can be recorded and/or controlled within the software.

Scientists at the Eppendorf Research & Development Lab in Connecticut, USA, have conducted CHO batch cell culture runs in which various sensors and control strategies were employed and compared. First, the capability of the control station to automatically detect and integrate sensors with ISM technology was utilised. Sensor health and maintenance was monitored using iSense software from Mettler-Toledo. In addition, the evo 200 (Fogale Nanotech) capacitance-based biomass sensor was also included for in-line growth monitoring.

The BioFlo 320 was used to control two batch suspension CHO cultures in a three-litre glass water-jacketed vessel. The runs differed in the automatic gassing strategy employed: one run used the three-gas algorithm and the other run used the four-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: an ISM polarographic DO sensor; an ISM optical DO sensor; and an analogue polarographic DO sensor. All three DO sensors were placed directly next to one another at the same height in the vessel.

Prior to autoclaving the vessel, an ISM gel-filled pH sensor was connected to the BioFlo 320 control station where it was automatically detected by the control station software, and calibration was performed. Unlike an analogue

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 USB. 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').

Reliable monitoring and gassing control

Both the three-gas and four-gas automatic DO control algorithms allowed the culture to reach similarly high viable cell densities. The four-gas experiment reached its peak cell density (8.89 x 10^6 cells/mL) sooner than the three-gas run (9.54 x 10^6 cells/mL). Glucose consumption and lactate and ammonia accumulation were comparable between the two cultures (Fig.1).

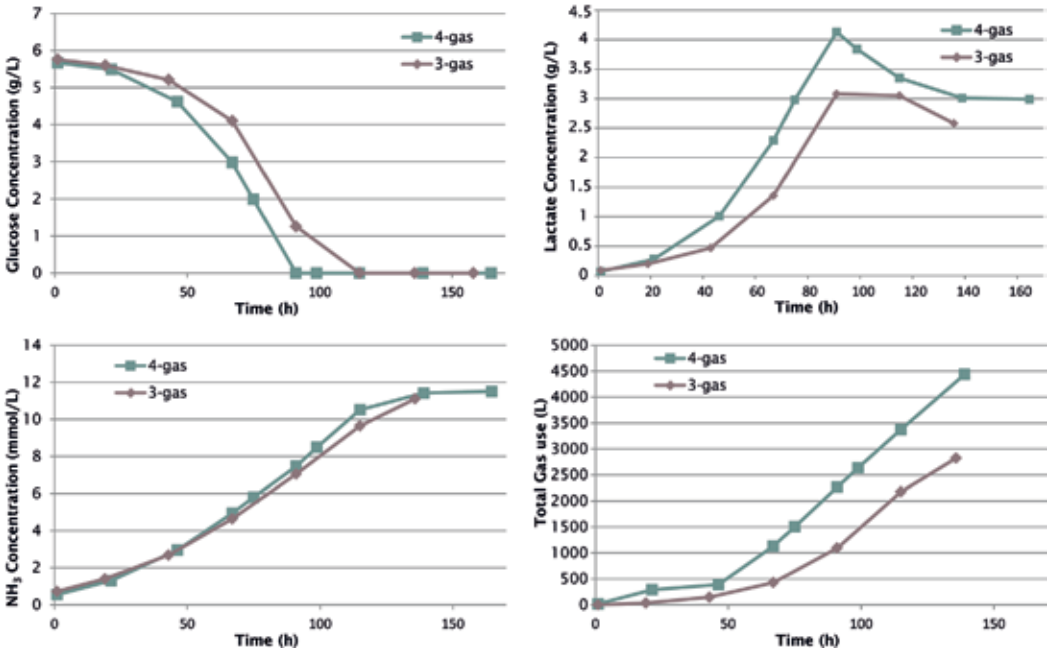
Consistent with the cell density trend, the three-gas culture consumed glucose slightly slower than the four-gas culture. When the glucose was exhausted, the cell growth and viability began to drop. Higher peak densities would have been possible if glucose and other necessary nutrients had been supplemented using a fed-batch protocol.

The two gassing control algorithms produced comparably healthy cultures, and showed some notable gas consumption differences. The four-gas culture consumed more gas overall, as illustrated in Fig.1, D. Since the three-gas algorithm does not utilise 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 four-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 three-gas or four-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. There was also a comparison made 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. No significant differences were seen between DO measurement by the three sensors.

With the use of intelligent sensors, the BioFlo 320 provides advanced process control for CHO cell culture. This method provided similar results using either the three-gas or four-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' greatly reduces operational risk due to potential sensor failure during a cell culture run.



Caption: Fig. 1. Comparing (A) glucose, (B) lactate, (C) ammonia concentrations, and (D) the total gas consumption between three-gas and four-gas during the bioreactor runs

Lessons learned as a result of these experiments

In these experiments, the ability to customise 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 bioanalyser, 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.

For more information ✔ at www.scientistlive.com/eurolab

Christiane Schlottbom is with Eppendorf in Europe. Stacey Willard & Ma Sha are with Eppendorf, Inc. in the USA. www.eppendorf.com