Optimizing Process Control In WAVE Bioreactor™ 20/50 System

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First published in March 2011
Introduction

- Disposable bioreactor technologies have been mainstream for over a decade, and like the traditional stainless-steel bioreactors, rapid progress has been achieved in the area of process control. This progress has been accelerated by the emerge of single-use or non-invasive probe technologies.

- Accurate and precise control over key parameters such as pH and dissolved oxygen (DO) is critical for cell cultivation. Toggling between CO₂ and base regulators is a common and unwanted problem that leads to oscillation and inaccurate control.
Introduction

• The new WAVEPOD™ II Integrated Controller has an improved control strategy for both pH and DO control. For increased usability it comes with an upgraded graphical user interface.

• A non-invasive optical pH sensor has also been developed to increase accuracy and reliability. It comes pre-calibrated and embedded into the Cellbag™ bioreactor.

• To facilitate real-time monitoring, the UNICORN™ DAQ 1.0 software has been designed for upstream application allowing key parameters to be monitored and managed subsequently.
Introduction

- The pH sensor together with the new control strategies will clearly show improved process performance.
- This study tested the performance of the new optical pH sensor and the new control strategies for pH and DO regulation were tested.

Fig 1. WAVE Bioreactor™ 20/50 System with WAVEPOD™ II.
Materials and Methods

- WAVE Bioreactor™ 20/50 System (GE Healthcare)
- WAVEPOD™ II (GE Healthcare)
- Cellbag™ 20L with optical pH sensor.
- CHO-K1SV cell line expressing monoclonal antibody IgG1.
- Power CHO-2 medium (Lonza) supplemented with Lucratone™ UF8804 (Millipore) and 6 mM L-Glutamine (GIBCO).
- UNICORN™ DAQ 1.0 software
Materials and Methods

- Cell density, viability, nutrients and physical parameters were measured off-line using the Bioprofile Flex (Nova Biomedical).
- IgG titers were determined with MabSelect SuRe™ analytical chromatography.
- pH was controlled by adding either CO₂ into headspace or base (7.5 % NaHCO₃).
- DO was controlled by adding pure oxygen into headspace on demand.
- Feeding of Lucratone™, glucose, glutamine and amino acids was carried out as needed.
- Temperature 37 °C, DO 30% air sat., pH 7.0, airflow overlay 0.2 L/min, rocking rate 25 and 6°rocking angle. Culture volume was 10 L.
Materials and Methods

This new pH control strategy introduces several new features such as:

• Adjustment of PID parameters for smoother pH control.
• Deadband for the acid/base controller is applied to the output of the regulator.
• Adjustment of delay time between CO$_2$ and base mode in CO$_2$/Base control.
• Automatic temperature compensation.
• Synchronized control and measurement cycle.
• LED current adjustment.
Materials and Methods

New DO control strategy introduces:

- New optical sensor board improve communication with PLC.
- PID controller with adjustable PID-parameters for DO control with \(O_2\).
- LED current adjustment.
Results

- The pH sensor displayed accurate and precise readings with stable regulation at a given setpoint. Three off-set calibrations were required during the cultivation that lasted for 15 days (Fig. 2).

Fig 2. Graph illustrating the pH value over time and the regulation of CO₂ and base. Blue arrows indicating time points of off-set calibration. Brown arrow indicating pH provocation with base. Data extracted from UNICORN™ DAQ.
Results

- The DO regulation was stable and responsive throughout the course of the cultivation. Drops in DO can be seen when the rocking was temporarily paused for sampling (Fig. 3).

**Fig 3.** Graph illustrating the DO value and O₂ regulation over time. Data extracted from UNICORN™ DAQ.
Results

- The effects on cell growth and viability were comparable except for the slight increase in viability between the pH regulated and non-regulated run (Fig. 4).

**Fig 4.** Cell growth and viability for the optical pH sensor-regulated culture run and the non-regulated run.
Results

- Regulation did, however, have significant impact on the product titer and was increased by 66% (Fig. 5).

![IgG titers](image)

**Fig 5.** IgG production titers for the optical pH sensor-regulated culture run and the non-regulated run.
Results

![Image: Optical pH sensor and process view in UNICORN™ DAQ.]

Fig 6. Optical pH sensor and process view in UNICORN™ DAQ.
Discussion

• The traditional deadband defining a lower pH value limit for the base addition, together with an inadequate control strategy, gives rise to insufficient regulation.

• By changing the deadband functionality for the acid/base controller and applying it to the regulator instead of the set point will lead to fewer fluctuations. With the latter type of deadband, the base pumps stops as soon as the deadband is reached and thus the set point is never reached. Eventually the fluctuations at the edge of the deadband will cause the errors in the controller to build up and generate pH overshoots.
Discussion

• Instead, the base is added intermittently by a pulse and the length of this pulse is calculated from the PI part of the PID regulator. If the length of the pulse falls within the deadband, the pulse is not executed. In regulators where only the P part of the PID regulation is active, the size of the error is not considered and will lead to error build-up.

• Adjustable delay time between CO$_2$ and base mode eliminates toggling between the different control modes.

• The transition delay gives input as to how long the controller must wait before changing from CO$_2$ to base mode and vice versa.
Conclusions

- The new non-invasive optical pH sensor and control strategies enhanced cell performance and IgG production.
- Readings from the new pH sensor were accurate and precise during the entire cultivation period.
- Stable regulation of both pH and DO without major fluctuations.
- No error build-up in the regulator.
- No toggling between CO₂ and base regulators.
Acknowledgments

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First published March 2011

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