

Evaluation of Finesse's Disposable TruFluor Optical Fluorescence Dissolved Oxygen (DO) and pH Sensors Compared to Existing Electrochemical DO and pH Sensor Technology in Mammalian Cell Culture Processes

Research Triangle Park, NC 27709

Eric Hickman, Engineer I, Cell Culture Development, RTP Pilot Plant
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Abstract

Biogen Idec is evaluating disposable biomanufacturing technology to create added flexibility. Considerable development work has been focused towards the implementation of disposable bioreactor systems as an alternative to industry standard stainless steel systems. Current electrochemical dissolved oxygen (DO) and pH sensor technology yields many disadvantages for current bioprocessing practices, such as high sensor cost, limited sensor re-usability or regular sensor maintenance, and sensor sterilization prior to use. Integration of non-intrusive phase fluorimetry based DO and pH sensors into single-use bioreactors could allow for a more complete disposable bioreactor system. Finesse TruFluor phase fluorimetry DO and pH sensors were implemented side-by-side with Mettler Toledo electrochemical DO and pH sensors in HyClone's (Thermo Scientific) Single Use Bioreactor (SUB) during multiple mammalian cell culture process runs. The findings of the evaluation will identify the likelihood of using fluorescence based sensors as an alternative to current electrochemical sensors for future bioprocessing purposes.

Introduction

Biogen Idec and Finesse Solutions have recently entered into a development agreement aimed at evaluating new technologies for single-use bioprocessing. As part of the agreement, Finesse has provided the RTP Pilot Plant with their new single-use optical probe technology: **TruFluor DO and pH**. The TruFluor sensors have been implemented with HyClone's (Thermo Scientific) Single-Use Bioreactors (SUB) for multiple mammalian cell culture runs and compared with conventionally used Mettler Toledo electrochemical DO and pH probes.

The TruFluor sensor platform is a single-use solution consisting of a disposable sheath, an optical reader, and a transmitter. The single-use sheath can be pre-inserted in a disposable bioreactor bag port, and irradiated with the bag, in order to both preserve and guarantee the sterile barrier. All wetted materials of the sheath are USP class VI compliant.

The optical reader utilizes an LED and a large area photodiode with integrated optical filtering, that minimizes photo-degradation of the acting sensing element. The design has been optimized to provide accurate in-situ measurement of dissolved oxygen using phase fluorometric detection in real time. The temperature measurement leverages a 316L stainless steel thermal window embedded in the sheath, and provides a highly accurate temperature measurement that can be used as a process variable.

TruFluor Sensor Components



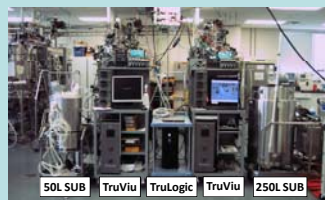
HyClone SUBs



1000L 250L 50L

Equipment Setup

50/250L SUB Systems with Finesse Controllers



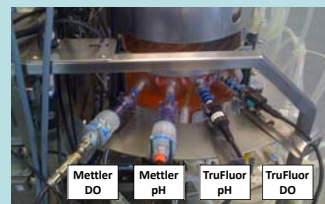
The 50L and 250L SUBs are controlled by DeltaV based TruLogic controller. Two TruViu RDPD systems interface with each SUB unit. Each TruViu system consists of an I/O tower, a Pump Tower, and a Gas Manifold. The TruFluor DO and pH transmitters are also integrated into the systems. The Finesse control system controls all bioreactor functions including temperature, agitation, pH, dissolved oxygen, gas overlay and gas sparge.

Mettler and TruFluor DO/pH Probes in 50L SUB

TruFluor DO/pH sensors were tested simultaneously with conventionally used Mettler Toledo DO/pH sensors in all SUB sizes (50L, 250L, 1000L).

Mettler Toledo DO/pH Sensors:

DO: InPro 6800 Series O₂ Sensor (P/N: 52200966) Length: 220mm, Diameter: 12mm
pH: 405-DPAS-SC pH Electrode (P/N: 104054481) Length: 225mm, Diameter: 12mm



1000L SUB Production Run



The 1000L glycol jacketed SUB is currently controlled using a 200L bioreactor skid. The 1000L SUB unit controls agitation while the 200L skid monitors and controls temperature, pH, dissolved oxygen, gas overlay and gas sparge. A TruFluor DO and pH unit have been integrated as stand-alone units used for monitoring and comparison purposes only. Development efforts with both Finesse (dedicated control system, sensors, etc) and HyClone (1000L bag design) are ongoing to develop an optimized 1000L SUB system that can sustain high productivity cell culture processes.

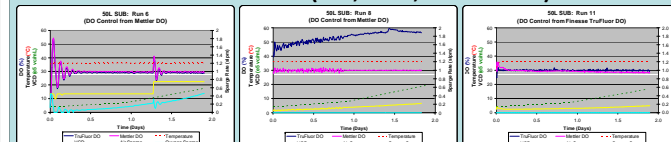
Experimental Method

DO Sensor Comparison: The TruFluor optical DO sensor was used 11 times in the 50L SUB, 4 times in the 250L SUB and once in the 1000L SUB. For the majority of cell culture runs, the TruFluor DO sensor has been used as a monitoring probe while the conventional Mettler Toledo DO sensor has been used as the controlling probe. In three 50L SUB runs, the TruFluor DO sensor was used as the controlling sensor and regulated the air/oxygen sparging as necessary.

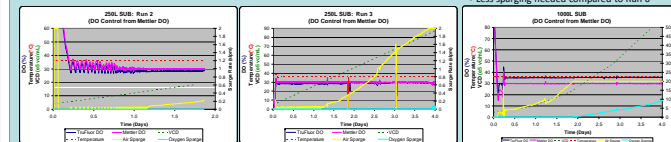
pH Sensor Comparison: The TruFluor optical pH sensor has been used once at the 50L and 250L scale. In both runs, the TruFluor pH sensor was used as the monitoring probe, with the Mettler Toledo pH sensor controlling CO₂ sparging and base additions for pH regulation

Experimental Results

TruFluor DO Sensor Results (50L, 250L, 1000L SUBs)

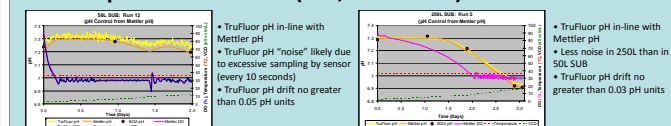


- Mettler DO set as controlling sensor
- TruFluor DO in-line with Mettler DO
- Faulty Mettler sensor w/bad calibration slope
- TruFluor DO value reflects correct DO value
- TruFluor DO set as controlling sensor
- Mettler DO in-line with TruFluor DO
- Less sparging needed compared to Run 6



- TruFluor DO in-line with Mettler DO
- TruFluor DO in-line with Mettler DO
- TruFluor DO offset 5% above Mettler DO
- Offset likely due to data collection scaling issue

TruFluor pH Sensor Results (50L, 250L SUBs)



- TruFluor pH in-line with Mettler pH
- TruFluor pH "noise" likely due to excessive sampling by sensor (every 10 seconds)
- TruFluor pH drift no greater than 0.05 pH units
- TruFluor pH in-line with Mettler pH
- Less noise in 250L than in 50L SUB
- TruFluor pH drift no greater than 0.03 pH units

Conclusions

As more cell culture processes move towards single-use process manufacturing, it is essential to use advanced process monitoring technology that performs as well or better than current standard technology by decreasing contamination risks, saving time and labor, and improving user operability.

TruFluor optical DO sensors provide a suitable alternative to conventional electrochemical sensors. They require minimal preparation time and are simpler to use than electrochemical DO probes as there are no membranes to replace and no electrolyte solution needed. Also, risk of culture contamination is decreased since the TruFluor optical sensors are non-product contact.

Limited testing has been performed with the TruFluor pH sensors due to development timelines. Like the TruFluor DO, the TruFluor pH sensor requires minimal preparation time and is very easy to use. In the two runs that have been performed, the TruFluor pH sensor reading has been within 0.05 pH units of the standard pH sensor.

Future Project Goals

- On-going single-use cell culture product development with Finesse Solutions includes:
- Further evaluate TruFluor pH sensors in all SUB volumes.
 - Monitor SUB process pressure by evaluating Finesse TruTorr pressure sensors
 - Evaluate control algorithms to improve future cell culture processes
 - Evaluate Finesse control system for 1000L SUB

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