Physical Chemical Sensors: Application to Bioreactor Monitoring and Control

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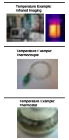
Introduction

Real-time bioprocess monitoring benefits: PAT Enablement for QbD

- maximize yield
- improve efficiency
- process reproducibility
- minimize costs
- reduce risk
- increase safety
- optimize product quality
- fully understand how a system works

Types of physical-chemical sensors

- electro-chemical
- optical
- radiofrequency based
- mechanical



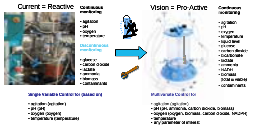
Criteria for monitoring bioreactors with physical chemical sensors

- minimally intrusive
- relatively inexpensive
- minimal to no calibration required
- sterlizable

New approaches to monitor bioreactors

- glucose
- dissolved oxygen
- pH
- dissolved carbon dioxide
- biomass sensing (dielectric spectroscopy)
- multiple parameters / bioreactor profiling
  - multivariate analysis (MVA)
  - near infrared (NIR) spectroscopy

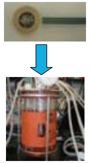
Achieving QbD Through Better Bioreactor Monitoring & Control

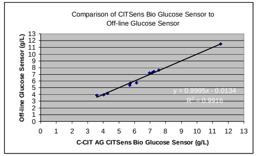


New Electrochemical Sensing Systems

Comparative performance of a wireless glucose sensor system (C-CIT AG) to off-line glucose (Nova Biomedical)

- 10 days (~234 hours) bioreactor run with CHO cells
- batch bioreactor run
- in-line sensors: dO, pH, temp, NIR, RF, glucose (C- CIT AG) exhibited linearity for 152 hours
- off-line systems: ViCell, Nova Biomedical (400, Flex)
- CITSens Bio data collection every 10 minutes; 4hr preconditioning in media prior to cell addition





dO_2 pH, dCO_2 Optical Sensing Systems

Optically-based fluorescent sensors (optodes)

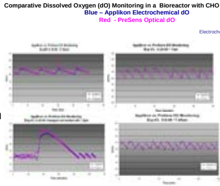
- dissolved oxygen
- pH
- dissolved carbon dioxide

Optode differences from electrochemical probes

- fluorophore required
- interrogating fiberoptic interface
- functional mechanism: luminescent lifetime decay

A. Comparative performance of an optical dissolved oxygen (dO) sensor system (Presens Precision Sensing, GmBH) to a polarographic dissolved oxygen probe (Applikon ez-Control Console)

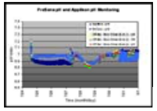
- Bioreactor calibrated with maximum air saturation of the cell media set at 100%
- Chinese Hamster Ovary (CHO) 0_2 consumption monitored over a three week period
- Optical dO sensor trended closely with the electrochemical dO probe.
- Both systems exhibited less than 1% drift and better than 0.5% sensitivity.



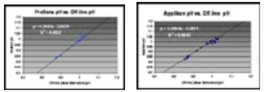
PreSens dO patches

B. Comparative performance of an optical pH sensor system (Presens Precision Sensing, GmBH) to an electrochemical glass probe. (Applikon ez-Control Console)

- Electrochemical glass probe precalibrated prior to sterilization; single point pH adjustment performed at start of the bioreactor run with off-line pH (Nova Biomedical)
- pH monitored over a one week period while growing Chinese Hamster Ovary (CHO) cells
- Optical pH sensor trended closely with the electrochemical pH probes both in-line and off-line.

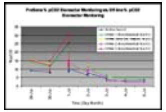


PreSens pH patches

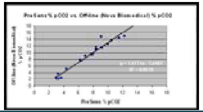


C. Comparative performance of an optical dissolved carbon dioxide (dCO_2) sensor system (Presens Precision Sensing, GmBH) to off-line dCO_2 (Nova Biomedical)

- Monitoring of dissolved carbon dioxide over an eight day period while growing CHO cells
- Optical dCO_2 sensor trended very closely with the standard electrochemical off-line dCO2 probes



PreSens dCO_2 patches



Radiofrequency Sensors: Viable Cell Density

Radiofrequency (RF) capacitance sensor for viable biomass

- Viable biomass important bioreactor parameter
- Admittance of an alternating current (AC) circuit measured

Comparison: optical versus RF probes for Biomass

- Optical probes typically monitor total biomass (both living and dead cells)
- RF impedance only counts living cells.
- Dead cells, lacking a selective permeable membrane barrier, do not act as capacitors since they do not exhibit a change in surface charge upon electric field application.
- Capacitance readings of living cells are sensitive to cell type and number.

RF probes for viable biomass in bioreactors

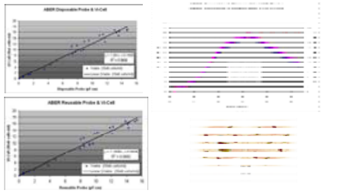
- Reusable Aber annular probe with four ring electrodes
- Aber disposable coupon with four flush platinum electrodes
- Both reusable Aber annular probe and Aber disposable coupon were incorporated into rigid-body disposable bioreactor

Reusable Aber annular probe

Aber disposable coupon

Comparative performance of reusable Aber annular probe to Aber disposable coupon in a disposable bioreactor controlled by Applikon ez-Control Console

- Monitoring of viable cell density over a two week period while growing Chinese Hamster Ovary (CHO) cells
- Both capacitance and conductivity measurements from both probes trended very closely to one another and to off-line measurements (Vi-Cell™ cell variability analyzer, Beckman Coulter).



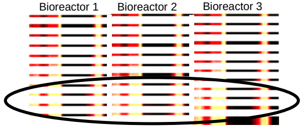
Near Infra Red (NIR)

Potential of near infrared (NIR) spectroscopy

- Bioreactor profiling
- Continuous bioprocess multiple analytes monitoring
  - Glucose
  - Glutamine
  - Lactate
  - Ammonia
  - Total Cell Density & Viable Cell Density

NIR spectroscopy to "fingerprint" a bioreactor run

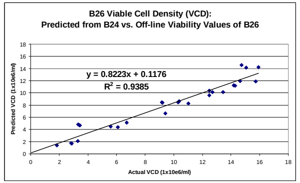
- CHO cells were grown in a benchtop Applikon bioreactor for 10 days.
- NIR spectra were taken using the (Thermo Fisher Scientific Antaris ® FT-NIR) every ten minutes.
- The processed spectra were plotted with Millipore in-house software in 3D depiction intensity maps which can serve as a "fingerprint" for subsequent bioreactor runs.
- Bioreactor run 1 exhibited detectable visual deviation from typical bioreactor runs 2 and 3.



NIR CHO Cell Bioreactor Run 3D Maps

NIR spectroscopy operational details

- Wavenumber ranges from 12000 to 4000 cm^{-1}
- Cell culture "soup" overtone and band combinations are used to correlate given spectra to analyte concentrations.
- NIR spectra correlated to independent analyte measurements through partial least squares (PLS) analysis. This generated "training" set (B24) is used to validate subsequent similar bioreactor runs (B26).
- This type of analysis can be applied to both simple a and complex parameters such as viable cell density



Near-Future Outlook

- The implementation of continuous multiple parameter monitoring is available multiple formats to suit reusable and single use bioreactors
- Advanced data collection and monitoring approaches will enable better bioreactor control and Quality by Design in bioprocess manufacturing.

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