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ABSTRACT

Single-use bioprocess bags were equipped with optical sensors for O₂ or pH. These sensors enable repeat, real-time, minimally invasive *in situ* measurements. The sensors are incorporated into the bioprocess bags by way of modified LDPE bag ports specifically designed for this purpose. Unlike traditional bag ports, the modified ports allow transfer of light, not fluid. Patches of sensor films known to be gamma-stable were applied to the surface of ports opposite a molded-in fiber optic connector. Once heat-sealed into bags in the same way as traditional fluid ports, bag sensors were interrogated from the bag exterior by connecting to an existing optical-based process monitor. Measurement of oxygen levels in such bags was demonstrated by both chemical and biological challenges, in both the aqueous and gas phases. Measured values spanned the range of 0-100% air saturation, and showed rapid response times. Optical pH ports were calibrated *in situ* against a traditional electrochemical pH meter, and showed a range of response of 3-4 pH units; detection range varied with sensor chemistry.

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Incorporation of optical sensors into bioprocess disposables promises to add to their attractiveness, by further in-lining measurement steps not traditionally amenable with the single-use paradigm. Both oxygen and pH sensors have been demonstrated.

MATERIALS & METHODS

Bag ports (figure 1) were molded of Class VI low-density polyethylene (LDPE). Films of sensor chemistry were heat-sealed onto the ports. Sensor-coated ports were heat-sealed into bags. A variety of bag formats and sensor configurations were made.

Sensors employed included Polestar[®] %p_{pm}-oxygen film, as well as two different pH films, with nominal response ranges of pH 4.0-7.0, 5.5-8.5. Oxygen films were calibrated and verified using either two concentrations (ambient or near-ambient and zero), or more thoroughly against a range of concentrations spanning the range of zero to ambient (air-saturated). The pH sensor ports were characterized against a traditional pH electrode across a number of pH values broader than the response range (e.g., pH 4-10 for the 5.5-8.5 sensor).

Cell culture experiments utilized CHO cells in HyClone SFM4CHO medium, supplemented with 10% HyClone Fetalclone I Bovine Serum. Cultures were analyzed offline for cell density using a CEDEX automated cell counting system (Innovatis, Inc.) and a Bioprofile 400 (Nova Biomedical) for pO₂, pH, and viable cell density.

Sensor Construction and Characterization

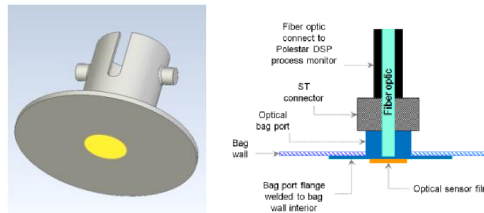


Fig. 1 (a) Engineering drawing of an optical bag port, showing a film of sensor applied to the surface which will wind up inside the bag. The features on the top are a fitting to connect a fiber optic cable. Sensors are made from gamma-stable Class VI materials. (b) Cross-sectional schematic showing an optical sensor port welded into a bioprocess bag and connected to a Polestar optical process monitor. Only the sensor film touches the bioprocess fluid; the film is optically interrogated from the outside, through the optical port.

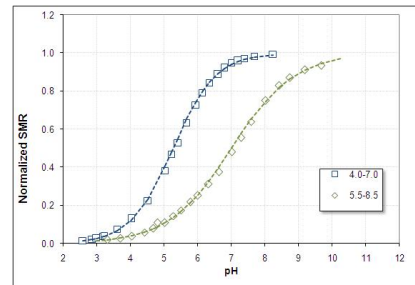


Fig. 2 Normalized responses of two of Polestar[®] pH sensors applied to bag ports, with nominal pH sensing ranges of -4.0-7.0 (blue) and -5.5-8.5 (green); these sensors show pKa values of -5.3 and 7.1, respectively. Plotted data points are individual experimental measurements of SMR, and the dotted lines are fits of the data to the Henderson-Hasselbalch equation, which describes the sigmoidal response of pH-related phenomena. Polestar[®] instrument measures SMR, or %signal magnitude ratio, which is the fluorescence emission at a pH-sensitive wavelength normalized to that of a pH-insensitive one. These calibration were done prior to incorporation into bags. Work is on-going to optimize sensor assembly and demonstrate utility in bags.

Rocker Bag Cell Culture Experiment

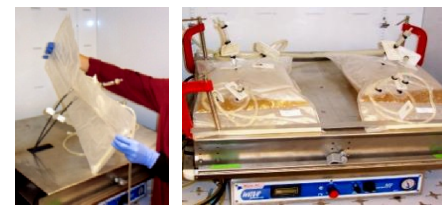


Fig. 3 Sensors were incorporated into the bottom surface of 2-liter culture bags for use on a Wave Bioreactor rocker tray. Apart from the need to construct an elevated platform to allow for attachment of optical cables to the sensors, the bags were utilized in the traditional fashion.

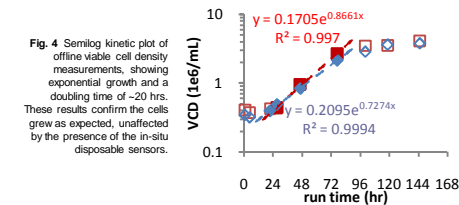


Fig. 4 Semilog kinetic plot of offline viable cell density measurements, showing exponential growth and a doubling time of ~20 hrs. These results confirm the cells grew as expected, unaffected by the presence of the in-situ disposable sensors.

(b) Correlation plot of online and offline pO₂ values.

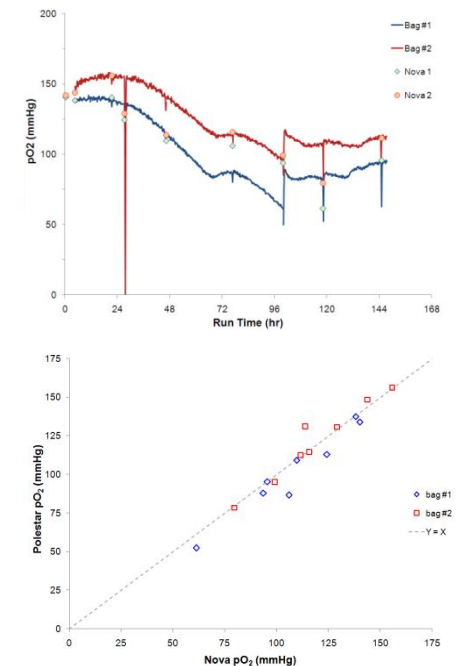


Fig. 5 (to the right) (a) Plot of in-situ measurements of pO₂ (continuous lines) across the duration of replicate 6-day CHO rocker bag cultures, along with periodic offline analyses (large individual data points) of the same. In-situ measurements were made via the optical sensor ports incorporated into the bag; readings were made every 10 minutes and logged to a PC. Offline measurements were made in a Nova blood gas analyzer. Sampling for off-line analysis required the rocking to be stopped, and for the sterile barrier of the bag to be broken to pull a sample. The down-spikes in the online pO₂ measurements correspond to sample times; for the sample at 27 hours, the rocker was accidentally left off for ~20 minutes, long enough for the cells to pull the pO₂ at the back bottom to zero.