Measurement and Control of Dissolved Carbon Dioxide in Mammalian Cell Culture Processes Using an in Situ Fiber Optic Chemical Sensor

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At high viable cell concentrations in large-scale mammalian cell culture processes, the accumulation of dissolved carbon dioxide (dCO_2 , typically quantified as an equilibrium gas-phase concentration) becomes problematic as a result of low CO_2 removal rates at reduced surface-to-volume ratios. High dCO_2 concentrations have previously been shown to inhibit cell growth and product formation in mammalian cells and to alter the glycosylation pattern of recombinant proteins. Therefore, reliable monitoring and control of dCO_2 are important for successful large-scale operation. Off-line measurements by instruments such as blood gas analyzers (BGA) are constrained by the low frequency of data collection and cannot be used for on-line control. In a preliminary evaluation of the YSI 8500 in situ sensor, a response time ($t_{90\%}$) of 6 min, sensitivity of 0.5% CO_2 (3.6 mmHg), and linearity of measurement ($R^2 = 0.9997$) between the equivalent gas-phase partial pressure of 0–180 mmHg (0% and 25% CO_2) were established. Measurements were found to be unaffected by culture pH and typical mammalian cell culture concentrations of glucose, glutamine, glutamate, lactate, and ammonium. The sensor withstood repeated sterilization and cleaning cycles. The reliability of this sensor was demonstrated in microcarrier-based Chinese hamster ovary (CHO) cell perfusion cultures at reactor scales of 30, 40, 340, and 2000 L and was successfully implemented in a dCO_2 control strategy using N_2 sparging.

Introduction

The detrimental effects of elevated dissolved carbon dioxide (dCO_2) concentrations (typically quantified as equilibrium gas-phase concentrations) on mammalian cell cultures are well documented (1–6). CO_2 is a non-polar molecule, which can diffuse across the cell membrane and lower the intracellular pH (7), thereby affecting the activity of intracellular enzymes and altering cellular metabolism (8, 9).

High dCO_2 has been shown to inhibit cell growth, nutrient utilization, and product formation and in some cases to alter protein glycosylation. Physiological dCO_2 in mammalian cells is roughly equivalent to a partial pressure (pCO_2) of 50–70 mmHg at 37 °C. Drapeau et al. (1) found that cell growth and specific M-CSF production rates in a CHO cell culture decreased by ~40% when the pCO_2 was increased from 53 to 165 mmHg. Gray et al. (3) observed a progressive inhibition of cell growth and product formation above a pCO_2 of 76 mmHg, in a CHO cell perfusion culture, with a 70% decrease in specific production rates at a pCO_2 of 148 mmHg. They attempted stripping CO_2 , as a means of controlling dCO_2 , by increasing the bubble size of the sparged oxygen gas. This strategy was constrained because of the difficulty in balancing O_2 supply and CO_2 removal. Taticek et al.

(6) reported that specific protein production, glucose and glutamine consumption, and lactate production rates of a baby hamster kidney (BHK) cell culture all decreased by ~40% as the culture pCO_2 was increased from 130 to 280 mmHg. Kimura and Miller (10) found that the proportion of sialic acids containing *N*-glycolylneuraminic acid decreased from 2.3–4.0% at a pCO_2 of 36 mmHg to 1.5–2.2% at a pCO_2 of 250 mmHg. We have observed that elevated dCO_2 concentrations can decrease product formation rates in microcarrier-based CHO cell perfusion cultures (results not shown).

To control pH, some mammalian cell culture processes employ the addition of base (e.g., NaOH), which can compound the detrimental effects of elevated dCO_2 . Kimura and Miller (4) found that the growth rate of a CHO cell line, compared to the control at a pCO_2 of 36 mmHg and an osmolality of 310 mOsm/kg, decreased by 30% with an increase in pCO_2 to 250 mmHg. A concurrent increase in osmolality to 376 mOsm/kg decreased the growth rate by 45%. In another study, the growth rate of a hybridoma cell line decreased by 40–50% when the pCO_2 was increased from 36 to 195 mmHg at a constant osmolality of 361 mOsm/kg (5). A concurrent increase in osmolality to 415 mOsm/kg decreased the growth rate by 63%. There was a 31-fold increase in cell death rate at a pCO_2 of 250 mmHg and an osmolality of 401 mOsm/kg.

There is a need to accurately monitor and control dCO_2 concentrations in mammalian cell culture processes. Off-line sensors are limited by the low frequency of data

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collection and biases introduced by sample handling and are difficult to use for continuous control. For these reasons, attempts have been made to develop in situ dCO₂ sensors.

Severinghaus and Bradley (11) developed the first in situ dCO₂ sensor by using a gas-permeable membrane to separate a bicarbonate buffer from the analyte solution. When CO₂ diffused across the membrane, a glass electrode measured the resulting pH change. The pH reading was converted to a dCO₂ output based on the chemical equilibrium:

where

$$K = \frac{[\text{H}^+][\text{HCO}_3^-]}{[\text{CO}_2][\text{H}_2\text{O}]} \quad (2)$$

The equilibrium constant, K , is constant at a given temperature. Since the concentrations of HCO₃⁻ and H₂O are significantly higher than those of H⁺ and CO₂, the concentration of CO₂ is proportional to the concentration of H⁺ (pH) for a constant temperature.

A sterilizable version of this sensor was constructed by Puhar et al. (12) for in situ use in fermentations. Shoda and Ishikawa (13) found that the internal bicarbonate buffer had to be replaced every 2 weeks and the probe itself, every 6 months, to ensure adequate measurement accuracy.

We evaluated a dCO₂ sensor based on this technology from Phoenix Electrode Corporation. Although the sensor response was adequate, we were uncomfortable with this probe for long-term use. A stainless steel plug was required to support the membrane during the steam-in-place process (SIP) and was then replaced with the nonsterile probe and electrolyte solution after the SIP. The inability of the electrode to be sterilized posed a potential contamination risk since sterility depended solely on the integrity of the membrane.

We also evaluated an infrared technology using attenuated total reflectance. The ReactIR 1000 midrange infrared (Mid-IR) sensor from Applied Systems, Inc. (ASI) had adequate response and sensitivity to dCO₂. The advantages of this technology were its robust construction and simultaneous in situ measurements of glucose, lactate, glutamine, glutamate, and ammonia, in addition to dCO₂. However, the labor-intensive calibration, high purchase cost, and the need for liquid N₂ cooling of the detector for adequate sensitivity made the implementation of this probe, solely for dCO₂ monitoring, impractical.

Several fiber optic dCO₂ probes have been developed recently, but few have been commercialized. Most of these sensors utilize the Severinghaus principle, with a pH-sensitive dye and optical fiber used in place of the pH electrode. The dye and bicarbonate buffer are contained in the distal end of the fiber, behind the CO₂ gas permeable membrane, where the optical fiber detects the relative fluorescence or absorbance of either the protonated, unprotonated, or both forms of the dye.

Although a number of fiber optic probes for dCO₂ detection have been developed (14–18), none were intended specifically for fermentation/cell culture applications. In many cases, the sensors had a short lifetime, were sensitive to interfering compounds, and had a restricted range of measurement.

Uttamlal and Walt (19) developed a fiber optic dCO₂ sensor using the fluorescent dye hydroxypyrenetrisul-

fonate (HPTS) for monitoring dCO₂ in fermentations. The advantage of this dye system was that the protonated and unprotonated forms had distinct excitation maxima, allowing a ratiometric measurement of pH and thus dCO₂. The use of a ratiometric method reduces potential error in the output due to photobleaching of the dye, lamp intensity fluctuations, or changes in the dye concentration. Furthermore, the isosbestic point of the dye (i.e., the wavelength at which the relative fluorescence of the two forms of the dye is the same) of 417 nm may be used to detect sensor drift due to photobleaching. YSI, Inc. has licensed Uttamlal and Walt's technology and has developed and commercialized a fiber optic dCO₂ sensor, the YSI 8500 (20). To our knowledge, this is the only available fiber optic, in situ sensor designed for industrial fermentation and cell culture processes.

In this study, we evaluated the YSI 8500 to determine whether it is suitable for continuous in situ monitoring and control of dCO₂ in mammalian cell culture processes. The sensor was tested against the following criteria: (1) adequate response time, sensitivity, and accuracy; (2) a linear range encompassing the operating dCO₂ range; (3) minimal effect by physical process parameters (pH and temperature); (4) minimal interference from medium components (glucose, glutamine, and glutamate) and metabolic wastes (lactate and ammonia); (5) tolerance to clean-in-place (CIP) and SIP processes; (6) adequate robust construction; (7) minimal contamination risk; (8) simple calibration process; and (9) minimal sensor drift. Following evaluation at reactor scales of 30, 40, 340, and 2000 L, we implemented the YSI 8500 in an on-line control strategy in which dCO₂ was removed by nitrogen sparging of the culture.

Materials and Methods

Spinner Flask Experiments. Experiments testing the sensor response time, linearity, sensitivity, and the effects of pH, temperature, metabolic nutrients and wastes, CIP conditions, and SIP conditions were conducted in a 250 mL spinner flask apparatus. This apparatus had 0.22 μm hydrophobic filters for gas supply and exhaust, a magnetic stir bar for agitation, and PHAR-MED L/S 13 tubing for gas sparging (i.d. = 0.8 mm). The spinner flask apparatus was placed in a warm box (Bellco Roll-In Incubator) at 37.0 °C (unless otherwise specified) and at atmospheric pressure (~760 mmHg). A magnetic stir plate (Thermolyne type 45700 Cellgro stirrer) agitated the spinner flasks at 100 rpm. The spinner flasks were filled with 500 mL of phosphate buffered saline (PBS), pH 6.8, to submerge the YSI 8500 probe.

Prior to the experiments, the YSI 8500 was calibrated between the range of 1% and 25% CO₂ by sparging a 25% CO₂/75% N₂ gas mixture until the output stabilized for a minimum of 30 min. YSI 8500 measurements were in units of % CO₂ and taken at 1-min intervals.

The desired dCO₂ concentrations were achieved by sparging the spinner flask with gas mixtures of known CO₂ concentration from certified (±0.2%), pressurized cylinders (Aga Gas) at approximately 100 cm³/min. For the sensitivity experiment, gases from 5% and 10% CO₂ pressurized cylinders were mixed by varying the relative flow through rotameters to generate the 5.6% and 6.2% CO₂ concentrations.

Two off-line blood gas analyzers (BGAs) (Ciba Corning 278 model and Chiron Diagnostics 248 model) were used to confirm the dCO₂ concentrations. Samples were taken from the spinner flasks for BGA analysis when the output

of the YSI 8500 had equilibrated for a minimum of 30 min. The BGAs were calibrated in the range of 0–10% CO₂. All experiments were performed in duplicate. pH measurements were made using a pH probe (Mettler Toledo type 405) and the BGAs (for pH 6.0–8.0).

The effect of cell culture metabolites experiment used the following chemicals: D-(±)-glucose (Sigma), L-(±)-lactic acid (Sigma), ammonium chloride (Mallinckrodt), 200 mM glutamine (Gibco BRL), and glutamic acid (Sigma). A stir/hot plate (Thermolyne Cimarec 2) was used to heat the spinner flasks to 50 °C for the effect of CIP experiment.

Cells and Media. A recombinant CHO cell line, grown on microcarriers in Genzyme's proprietary cell culture medium, was used in this work. Initially, the cell culture medium contained phenol red. By the end of the process, the phenol red was completely removed.

Bioreactor Cell Culture. The working volumes of the reactors used in this study were 30, 40, 340, and 2000 L. Prior to inoculation, the reactors with the probe in place and containing PBS were steam-sterilized at 134 °C for a minimum of 40 min. The cell culture was operated in a continuous, perfusion mode. Bioreactor pressure was controlled to 3.0 psig. N₂ was sparged into the cell culture through a sintered sparge element on a dip tube.

Calibration and Operation of the YSI 8500. Prior to the SIP process, a new sensor capsule was placed onto the distal end of the 25 mm probe for the 30, 40, and 2000 L scales and onto the 19 mm probe for the 340 L scale. The probe was inserted into the reactor and sterilized. After the SIP step, the reactor temperature and the agitation rate were controlled to setpoint and the reactor was sparged with a 15% CO₂/85% N₂ gas mixture. This gas mixture was generated either by using a premixed gas mixture from a pressurized gas cylinder (Aga Gas) or by using the bioreactor's calibrated mass flow controllers (MFC). During the calibration and the cell culture process, the YSI 8500 measured the dCO₂ at 1-min intervals in units of % CO₂. The calibration of the YSI 8500 was performed when the output of the YSI 8500 was stable for at least 30 min.

Results and Discussion

Sensor Response Time. The response time of the probe was tested by removing the probe from a 250 mL spinner flask equilibrated to 5% CO₂ and quickly inserting it in another spinner flask equilibrated to 10% CO₂. The response time was defined as the time it took for the sensor to reach 90% of its final dCO₂ value ($t_{90\%}$). The $t_{90\%}$ of the YSI 8500 to a change from 5% to 10% CO₂ was found to be 6 min (Figure 1). This response time is adequate, considering the slow metabolic rates in mammalian cell culture processes.

Linearity and Range. To test the linearity of measurement in the dCO₂ range of 0–25%, which encompasses the pCO₂ of typical mammalian cell culture processes (100–200 mmHg), the dCO₂ in the spinner flask was increased incrementally from 0% to 5%, 10%, 17%, and then 25% CO₂. The output of the YSI 8500 was linear in this dCO₂ range with an $R^2 = 0.9997$ (Figure 2). BGA measurements were also linear in this range ($R^2 = 0.95$), despite the instruments having been calibrated in the range of 0–10% CO₂. The maximum CO₂ concentration that may be used for the calibration of the BGA 278 model is 12%.

Sensitivity. The sensitivity was defined as the ability of the sensor to detect slight variations in dCO₂. To test the sensitivity of measurement, the dCO₂ concentration

Figure 1. The response time ($t_{90\%}$) of the YSI 8500 from 5% to 10% CO₂ at 37.0 °C in PBS was found to be 6 min.

Figure 2. Linearity, from 0% to 25% CO₂, of the dCO₂ output of the BGA and YSI 8500 at 37 °C. The dCO₂ calibration range of the BGA is 0–10% CO₂. The dCO₂ calibration range of the YSI 8500 is 0–25% CO₂.

in the spinner flask was incrementally increased from 5.0% to 5.6% and then 6.2%. Both the BGA and the YSI 8500 were able to detect dCO₂ changes of approximately 0.5% CO₂ (~3.6 mmHg) (Figure 3).

Effect of pH. Since the output from the YSI 8500 is a function of the pH in the sensor capsule, it was important to establish the effect of changes in culture pH on the measured dCO₂ values. To test the effects of pH changes, the PBS contents of the spinner flask, initially lowered to pH 2.0 with 1.0 N HCl, were incrementally increased to pH 8.0 with 2.0 M NaOH. The dCO₂ was maintained at 10%. Under these conditions, dCO₂ measurements were found to be unaffected by pH changes (Figure 4).

Effect of Metabolic Nutrients and Wastes. The interference of common metabolic nutrients and wastes, individually and in combination, on the measured dCO₂ output of the YSI 8500 was tested at ranges typical of mammalian cell culture processes: glucose 0–7 g/L, lactate 0–5 g/L, glutamine 0–5 mM, glutamate 0–5 mM, and ammonia 0–5 mM. Desired concentrations were achieved by adding aliquots of concentrated solutions or a measured amount of the chemical to the spinner flask, with the contents of the spinner flask maintained at 10% CO₂. Since none of the metabolites, either individually or a combination of all five compounds, noticeably affected the dCO₂ measurement at the upper and lower limits of the test concentration ranges, intermediate concentrations were not tested.

Effect of Clean-in-Place (CIP) and Steam-in-Place (SIP) Processes. Upon termination of most mammalian

Figure 3. Sensitivity of the dCO_2 output of the YSI 8500 and the BGA at 37.0 °C.

Figure 4. Effect of culture pH on the dCO_2 output of the YSI 8500 and the BGA at 37.0 °C in PBS. The dCO_2 (10% CO_2) was constant for the duration of the experiment.

cell culture processes, the bioreactor, generally with all in situ probes still inserted in the reactor, undergoes a CIP process. The ability of the sensor capsule to withstand a typical CIP process was tested by exposing the probe to a 0.2 M KOH solution at 50 °C for a minimum of 4 h. Prior to the CIP process, the accurate measurement of dCO_2 in PBS equilibrated to 10% CO_2 was ensured. The PBS was then removed from the spinner flask and replaced with 0.2 M KOH. After the CIP process and following a triplicate rinse with water-for-injection (WFI), the flask was refilled with 500 mL of PBS. Measurements taken when the PBS was equilibrated with 10% CO_2 remained accurate, indicating that the sensor was not affected by the CIP process (% difference pre-CIP $\rightarrow$ post-CIP = 2.5%).

The ability of the sensor capsule to withstand the high temperatures of a typical SIP process was investigated during an autoclave cycle of 20 min at 122 °C. Prior to the SIP process, the accurate measurement of dCO_2 in PBS equilibrated to 10% CO_2 was ensured. After the autoclave cycle, the spinner flask was reequilibrated with 10% CO_2 at 37.0 °C. The dCO_2 measurements remained accurate, indicating that the sensor was not affected by the SIP process (% difference pre-SIP $\rightarrow$ post-SIP = 1.5%).

Calibration of the YSI 8500. The calibration period was observed to be approximately 4 h at the 30 L scale (Figure 5). The length of the calibration period is primarily a function of the agitation rate, the gas sparge flowrate, and the mass transfer characteristics of the bioreactor (e.g., the bubble size), rather than that of the sensor response. At the 30 L scale, we did not optimize

Figure 5. Calibration of the YSI 8500 in a 30 L reactor at 36.0 °C. During the calibration period, a gas mixture of 15% CO_2 was sparged through a sintered tip element at a flowrate of 1 sL/min in ~ 25 L of PBS. At 4 h, the output of the YSI 8500 had stabilized. At 5 h, the output of the YSI 8500 was set to 15% from $\sim 15.5\%$ CO_2 (the equilibrated value).

these parameters for minimal calibration time. During the calibration period, a gas mixture of 15% CO_2 was sparged through a sintered tip element at a flowrate of 1 sL/min into ~ 25 L of PBS (initially at a dCO_2 of 0%). At 5 h (Figure 5), the output of the YSI 8500 was set to 15% from $\sim 15.5\%$ CO_2 (the equilibrated value).

The calibration period could be shortened if the probe could be calibrated in an external device of less volume (e.g., the spinner flask apparatus) prior to the SIP process.

Effects of Bioreactor Pressure and Temperature on Measured dCO_2 . In mammalian cell culture processes, it is conventional practice to quantify dCO_2 as an equilibrium gas-phase concentration either as a mole fraction (y_{CO_2}) or as a partial pressure (pCO_2). The YSI 8500 currently uses % gas-phase concentration as the standard unit of measurement to allow for an easy calibration with a gas of known CO_2 composition at any given condition. The detrimental effects of elevated dCO_2 concentration to mammalian cell cultures presumably are better correlated with the liquid-phase concentration. If temperature and pressure are kept constant, the gas and liquid-phase concentrations are directly proportional, in which case either may be used to monitor and correlate dCO_2 to physiological effects.

In large-scale reactors, the hydrostatic pressure can have a significant effect on the dCO_2 (e.g., 1 m of liquid height above the probe increases the pCO_2 by approximately 75 mmHg). From the literature and our observations, this change can be physiologically significant. Even fluctuations in barometric pressure can change the pCO_2 by ± 10 mmHg.

If temperature is varied during the process, then a true liquid-phase concentration or an "effective gas-phase concentration", corrected to account for differences in solubility, would be more appropriate. With an increase in temperature from 30 to 40 °C, the solubility of CO_2 decreases by approximately 25%. This would be equivalent to an increase in "effective" pCO_2 from, for example, 100 to 125 mmHg at 37.0 °C and may be physiologically significant.

The effect of temperature changes on measured dCO_2 values was determined by incrementally increasing the temperature of the spinner flask contents from 29.0 to 41.0 °C, while maintaining a constant dCO_2 of 10%. The chosen temperature limits encompass the typical range of mammalian cell culture processes. It has been shown

Figure 6. Effect of temperature on the dCO_2 output of the YSI 8500 and the BGA. The dCO_2 of the PBS contents was constant for the duration of the experiment at 10% CO_2 . The YSI 8500 and the BGA data were temperature-corrected.

that heat shock results in the cessation of cell growth in mammalian cells at temperatures greater than 41 °C (21) and that the growth of mammalian cells essentially stops at 29 °C (22).

Since the saturation concentration of dissolved gases is a function of temperature, the YSI 8500 has an internal algorithm to correct measured values at different operating temperatures. This temperature compensation algorithm was tested and found to be accurate (Figure 6). The BGAs used in this experiment measured the pCO_2 at 37.0 °C and corrected for the temperature of the reactor sample (29–41 °C) using an operator-entered sample temperature and an internal algorithm. However, the corrected values from the BGAs were inaccurate, possibly as a result of an inaccurate algorithm, as compared to those from the YSI 8500 (Figure 6).

Sensor Drift in Long-Term Culture. Since continuous mammalian cell culture processes can be operated over extended periods of time, the output of the YSI 8500 was monitored to determine whether it significantly drifted during the process. The drifting of the output of the YSI 8500 was determined by comparison with the measured value from the BGA, which is less susceptible to drifting since it is calibrated with certified gases every hour. Over the approximate 2-month duration of a cell culture run, there was a relatively constant offset between the BGA and the YSI 8500 of 29 ± 5 mmHg (Figure 7). A constant offset suggests that there was no significant drifting in the YSI 8500 measurements. After a 35-day run at the 40 L scale, a postrun calibration was performed with 10% CO_2 (87 mmHg); it was found that the sensor had drifted by approximately 10 mmHg over this time period.

The difference between the outputs from the BGA and the YSI 8500 was minimal when both instruments measured dCO_2 at atmospheric pressure, in the absence of cells, and at 37.0 °C. The approximately 20–30 mmHg difference between the outputs from the BGA and the YSI 8500 (Figure 7) most likely arose from sample off-gassing, which can occur by the change in total pressure (atmospheric, gauge, and hydrostatic head above the probe) from the pressurized bioreactor to the sample bottle at atmospheric pressure. The extent of off-gassing is dependent upon the time period from reactor sampling to actual BGA sample injection for analysis. Other factors that could have led to this measurement discrepancy are heterogeneous mixing between the probe and sample port location and high viable cell concentrations (i.e., cellular respiration may increase the pCO_2 in the sample).

Figure 7. Comparison of the YSI 8500 and BGA dCO_2 measurements in a 340 L microcarrier-based CHO cell perfusion process.

Figure 8. Controlling the pCO_2 under a process limit of 110 mmHg by adjusting the N_2 sparge flowrate on the basis of the on-line output from the YSI 8500 in a 2000 L microcarrier-based CHO cell perfusion process.

Controlling dCO_2 Using N_2 Sparging. Once the reliability of the YSI 8500 had been established, a dCO_2 control strategy was investigated. Using a predetermined upper pCO_2 process limit and the on-line output trends from the YSI 8500, the N_2 sparge flowrate was increased if the pCO_2 was increasing, maintained if the pCO_2 was constant, or lowered if the pCO_2 was decreasing. The N_2 sparge flowrate adjustment could be varied to respond to an increasing/decreasing trend as indicated by the pCO_2 probe output. Manual control was monitored every 3 h to maintain the dCO_2 below the upper process limit. Most large scale mammalian cell culture processes operate within the pCO_2 range of 150–200 mmHg. For the 2000 L CHO cell microcarrier perfusion process, an upper pCO_2 process limit of 110 mmHg was chosen to test the control method (Figure 8). For this 20 day run, the dCO_2 was controlled in the operating range by adjusting the N_2 sparge flowrate. This method was successful in maintaining the pCO_2 below the upper process limit. Future work will focus on configuring an automatic proportional-integral-derivative (PID) control loop to control the pCO_2 to a setpoint. The dissolved oxygen (DO) was maintained at setpoint ($\pm 1\%$ air saturation) for the entire duration of this run (results not shown). The DO control mechanism at the 2000 L scale consisted of in situ DO probes, which were linked to a controller that varied the magnitude of the oxygen sparge flowrate. During periods of increasing N_2 sparge flowrate, the DO control compensated for increased CO_2/O_2 removal by increasing the O_2 sparge flowrate. At no point in this

experiment was increased foaming due to the N₂ sparge flowrate observed.

Conclusions

The fiber optic based dCO₂ sensor, the YSI 8500, was determined to meet all selection criteria for in situ implementation into our bioreactors. The robust construction of this probe posed a minimal risk of process contamination. The response time, $t_{90\%}$, of 6 min was adequate for the typical rate of change in the dCO₂ of the bioreactor and for on-line control. The range of the sensor was at least 0–180 mmHg, which spans most mammalian cell culture dCO₂ operating ranges. The output was linear ($R^2 = 0.9997$) over this range. Sensitivity of measurement was 0.5% CO₂ (3.6 mmHg). The presence of metabolic wastes (lactate, glutamate, and ammonium) and nutrients (glucose and glutamine) as well as physical process parameters (pH and temperature) did not interfere with the accurate measurement of dCO₂.

There was no significant drift or loss of accuracy due to sensor drifting at reactor scales of 30, 40, 340, and 2000 L for a 2-month duration. A constant offset of approximately 20 mmHg was observed between the measurements of the YSI 8500 and the off-line BGA for the entire length of the process. This offset is attributed to bioreactor sample off-gassing prior to BGA analysis; thus, we believe the in situ probe gives a more reliable indication of reactor conditions. The YSI 8500 is amenable to continuous monitoring and feedback control. A dCO₂ control strategy, using N₂ sparging to maintain the stability of the cell culture at different reactor scales, was demonstrated.

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