HAMILTON

DISSOLVED CO_2 SERIES:

Are Current Dissolved CO_2 Measurement Technologies Good Enough?

White Paper

Table of Contents

Abstract

Dissolved CO_2 has been shown to be a Critical Process Parameter (CPP) in biopharma processes. Measurement and control of this parameter can be highly beneficial to process improvement, including increased product yield and quality. There are several common ways of measuring CO_2 in the bioreactor, each with benefits and deficiencies. This white paper discusses the factors that should be considered when choosing a CO_2 measurement method and the major values and issues with each method in relation with their use for bioprocess control. This paper also describes calculations using a soft sensors approach, off-line analyzers such as Blood Gas Analyzers (BGAs), on-line measurements in the form of off-gas sensors, in-line measurements using the Severinghaus principle, and the potential for other technologies, such as optical/spectroscopic.

Keywords:

Dissolved Carbon Dioxide, CO_2, pCO_2, Bioprocesses, Bioreactor, PAT, In-line Measurement, In-situ Measurement, On-line Measurement, Off-line Measurement, Critical Process Parameter, Real-Time Control, Severinghaus, NDIR Spectroscopy, Soft Sensors, Off-Gas Analysis, BGA

1. How Should Dissolved CO_2 be Monitored According to PAT Principles?

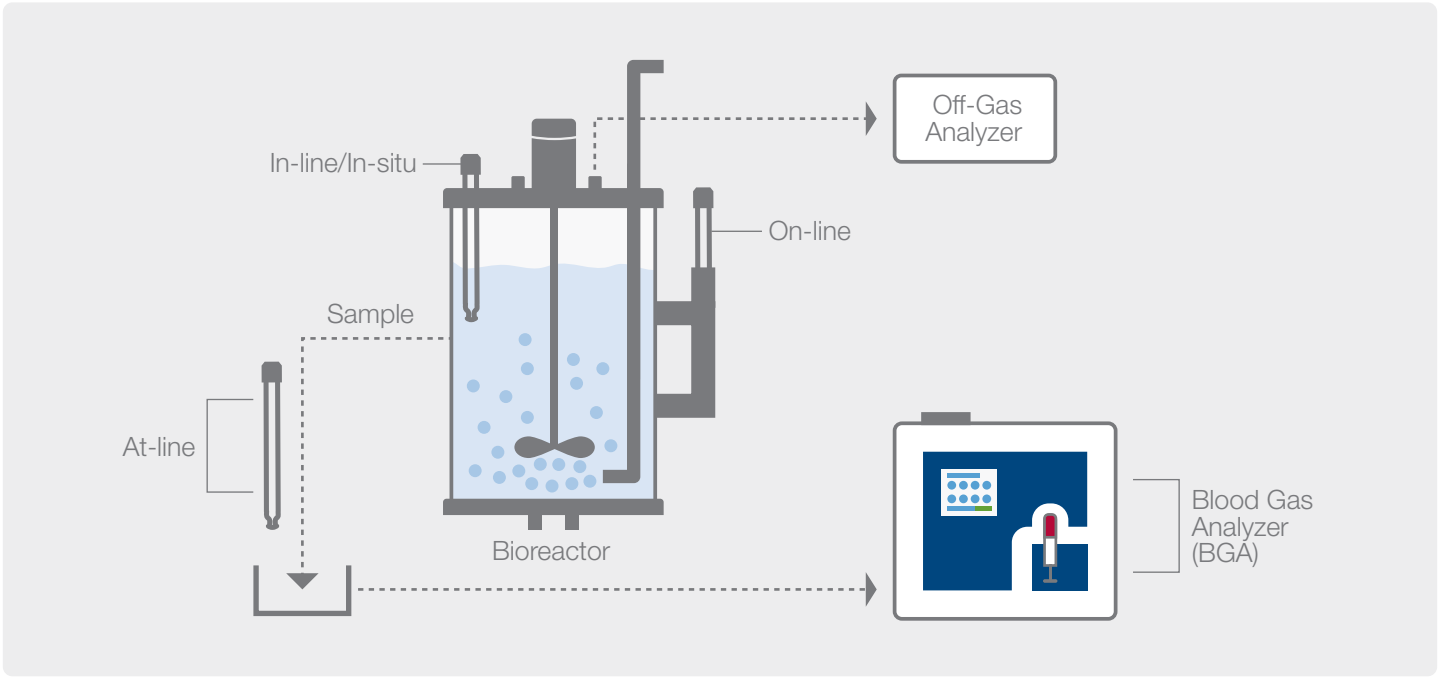
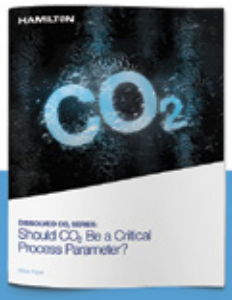


Figure 1: Different methods of process monitoring according to the PAT guidance (2004)[1] and later scientific literature.[B]

CO_2 affects many aspects of a typical bioprocess. As both a product and reactant of metabolism and an integral component of commonly used buffer systems, it is critical to control at optimal levels. Doing so has been shown to substantially increase product yield and product quality. In order to determine the optimal dissolved CO_2 (DCO_2) profile for a process, in-line measurement is required to provide continuous data from the reactor. Development of this optimal profile (or golden batch) at the R&D level enables maximum productivity and quality in manufacturing. DCO_2 is a Critical Process Parameter (CPP) as defined by the Process Analytical Technology (PAT) guidelines from the FDA.[1] As a CPP, it should be controlled in real time to improve bioprocess performance. Pairing of in-line measurement with automated control during the scaling up of a process is the key to process optimization and reduction of scale-up/scale-down iterations. For a complete introduction to the importance and benefits of measuring and controlling DCO_2, please see Part 1 of Hamilton's Dissolved CO_2 White Paper Series: "**Should CO_2 Be a Critical Process Parameter?**"[A]



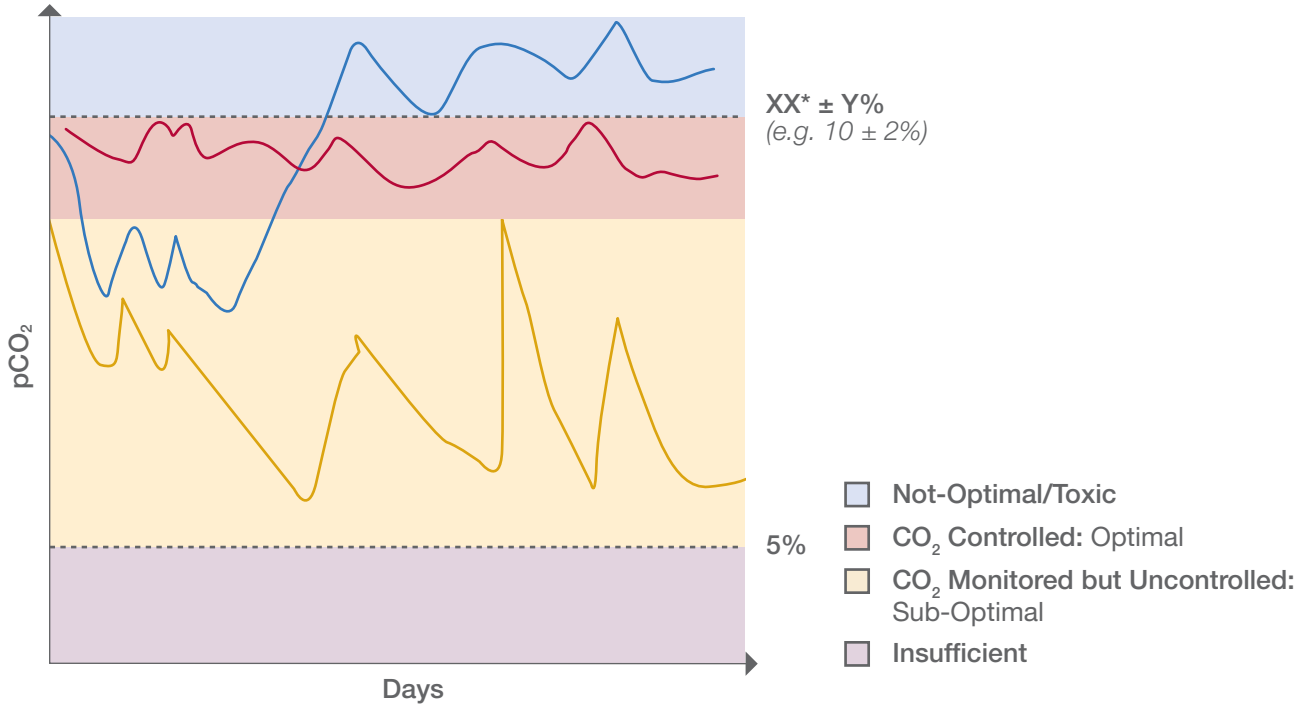
For more information and examples on the value of in-line DCO_2 measurement,
Click Here to Download Part 1 of the Dissolved CO_2 White Paper Series.[A]

There are several different commonly used approaches to measuring DCO_2 in the bioreactor. This white paper will outline this variety including those used off-line, on-line, and in-line.

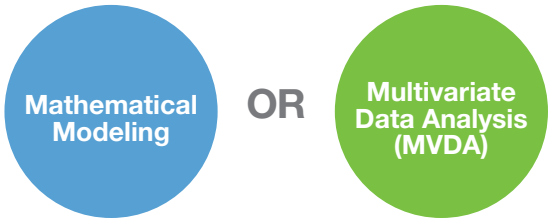
When determining the best measurement technology for an application, there are several factors that need to be considered. As with any analytical device, the ideal DCO_2 sensor should be accurate and precise at a reasonable cost. The working range needs to encompass typical culture requirements, for example between 30 and 110 mmHg (50–150 mbar) for mammalian cultures. The lifetime of the technology is also very important. A device is of limited utility if it cannot withstand an entire bioprocess run, including cleaning such as autoclavation, Sterilization In Place (SIP), and/or Cleaning In Place (CIP) cycles. One of the most difficult requirements of process analytics devices is selectivity. CO_2 has very similar properties to other volatile substances present in bioreactor, so it is of critical importance that the selected measurement is insensitive to other analytes. Determining whether a measurement should be done off-line, on-line, in-line, or some combination is an important decision that is too often out of the hands of the users. For example, many metabolites would be valuable to measure in-line, but at present, no technology is widely accepted as capable of achieving such a measurement in bioprocesses.[B] DCO_2 has options for each implementation, and the benefits and deficiencies are described below. In order to fully achieve all the benefits DCO_2 monitoring can offer however, it at least needs to be measured in-line and automatically controlled off of if possible (see example below). This has been demonstrated at length in the scientific literature, the first volume of this white paper, and is in accordance with the PAT guidelines. From a practical perspective, an appropriate DCO_2 sensor should have dimensions and sterilizability befitting the application in addition to a usable response time. Finally, an ideal sensing technology would have low maintenance requirements and a robust measurement not prone to error, including the retention of precision and accuracy throughout multiple processes.

There is no perfect technology for the measurement of DCO_2 that meets all of these needs for typical biopharma applications. This white paper aims to provide a guide to the values and drawbacks of the several measurement options available including soft sensors, off-line analyzers, on-line, off-gas analyzers, and in-line sensors.

Example: Simplified example about how to exploit pCO_2 real-time control in order to maximize the bioprocess productivity.[A]



2. Indirect Measurement Through Derivation (Soft Sensors)



Mathematical modeling or Multivariate Data Analysis (MVDA) can be used to approximate a measurement in the absence of a physical reading. Because of that they are often referred to as “software sensors” or “soft sensors." Mathematical modeling involves predetermined and fixed mechanistic equations, while MVDA makes use of continuously learning algorithms based on statistics. Both approaches utilize multiple variables to create a model calculation. In order to approximate dissolved carbon dioxide (DCO_2) in the bioreactor media, many factors need to be taken into consideration, especially if the model will be used in scale-up experiments.[2] Gas laws such as Henry’s law

Henry’s Law Equation:

$$H_{CO_2} = \frac{C_{CO_2L}}{P_{CO_2}} \left[\frac{mmol}{L\ bar} \right]$$

are insufficient compared to the complexity of DCO_2 interactions in the reactor, so more complex modeling is required. Parameters that are typically considered in MVDA calculations of DCO_2 may include off-gas CO_2 measurements, in accordance with an understanding of Henry’s law. To implement this factor successfully, the phase transfer of CO_2 from the liquid media to the gaseous headspace needs to be modeled.[3]

Data from an in-line pH sensor are used due to the impact of CO_2 on pH and its involvement in the buffer system.[4]

Any parameters that could impact mass transfer such as buffer and medium characteristics need to be included as well. Finally, any available information about the cells should be included. Respiratory data, oxygen levels, and other information directly related to the viable cell population should be factored into these calculations.[5]

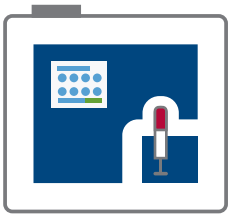
In some applications, MVDA or mechanistic calculations may be the best understanding of DCO_2 that is feasible, but the deficiencies of such an approach should be considered. The primary deficiency is the complexity and the effort required to identify and maintain them. There are a multitude of factors that contribute to these calculations that each have an associated margin of error. The resultant calculation can only then be as accurate as the cumulation of these errors. This calculation is also extremely matrix-, and therefore application-dependent. A cell culture or fermentation is a living process that is constantly changing. Despite best efforts towards constant conditions and a stable environment, it is well understood that the contents of a reactor change throughout the process, and necessarily so. Soft sensors can be well suited for established processes that are very well characterized, but such environmental changes make it extremely difficult to accurately determine the DCO_2 of a reactor throughout a process. In some cases, these calculations may be done in a way that is timely enough to implement a control strategy, even if utilizing manual adjustments, but the reliability of the calculations should call into question the wisdom of doing so. The limits of deriving a Critical Process Parameter such as dissolved CO_2 from other parameters are perhaps most clear when used for control in Design of Experiments (DoE) because the CPPs are varied heavily in the development of the golden batch (sometimes referred as “gold standard”) profiles.[6] When feasible, a matrix-independent technology is always a more reliable and often a more accurate choice.

3. Off-line and At-line Measurement with Reference Analyzers

A relatively common approach to measuring CO_2 from the bioreactor is to use a standalone Blood Gas Analyzer (BGA).[7] BGAs are especially common at the R&D and laboratory scale as they can be used as a reference tool for many parameters. They can also be located in production areas, e.g. for at-line analysis, but less commonly. The scarcity of BGAs in production is due to the fact that they take up considerable space and have to be compliant with all cleaning and compatibility rules of the production facility. Maintaining sterility throughout the entirety of the complex instrument can be extremely cumbersome, especially when including sampling procedures and maintenance activities. It is much more common to sample in the production area and bring the sample and probe to the laboratory for an off-line check, although use of a sampling probe with automated at-line analysis is also possible. The off-line check gives an acceptable, though error-prone measurement that can be used as a reference analytical method, but this complexity is too high for real-time control as detailed in this chapter.

Off-line benchtop analyzers provide measurements without the need for additional ports on the bioreactor. This is highly beneficial because having a limited number of ports and/or limited space on the headplate is a leading reason for omitting beneficial process analytics. In addition to measuring CO_2, many BGAs may be coupled with other technology that can analyze pH, DO, metabolites, and even cell numbers such as viable cell density in parallel. In this case, the BGA is one of the technologies embedded in the multi-purpose off-line/at-line analyzer. When there is overlap with in-line parameters (often pH and DO), those analyzers can be used as a reference tool. Another benefit of using them is that a single device can serve multiple bioreactors, although this includes some limitations in regard to the availability for many samples and running cost (discussed further below). However, this approach should be taken with caution as there are several deficiencies and sources of error.

One of the largest contributions to discrepancies between in-line sensors and the BGA analysis comes from sample handling. This is especially true for CO_2 and other volatile analytes. Sampling inherently leads to changes in sample pressure and temperature that will impact the CO_2 measurement. Because DCO_2 is commonly measured



BGA Highest Accuracy in Measuring Ranges

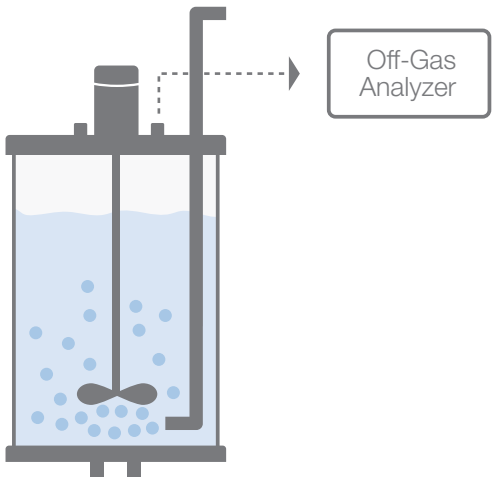
±5 mmHg between 30–60 mmHg

as partial pressure, differences in temperature and total pressure of the system can lead to significant differences between in-line and off-line measurements. As the name suggests, Blood Gas Analyzers were originally used to analyze pO_2 and pCO_2 in blood samples. Such devices exhibit their highest accuracy in measuring ranges close to human physiology (e.g. ±5 mmHg between 30–60 mmHg), while at higher measuring range, such as 100–200 mmHg the measuring bias can be up to ± 15 mmHg.[8] As mentioned, analyzers such as these are typically used for off-line measurements where a sample is taken manually and brought to the analyzer. Some groups may use automated sampling (i.e. an at-line method) to lessen sampling bias, time, and labor, but there are still drawbacks to this approach.

Regardless of the off-line or at-line configuration, a multipurpose analyzer can only be used for a relatively low frequency of discrete data points. It is very difficult to establish a control method using this data, and the continuous automated control directed by the PAT initiative is almost impossible. Increasing sampling frequency is somewhat unrealistic, especially in smaller reactors, due to the volume removed with each sample. This is true even in the case of ultrafiltration and dialysis sampling probes where the sampling volume is relatively low. The analyzers also tend to have high initial cost and very high maintenance costs as the result of expensive and consumable analysis cartridges. The cost is prohibitively high to make measurements frequent enough for control. From an analytical and statistical perspective, a measurement is required to be performed in triplicate to be considered reliable. For all the reasons discussed here, it is common to find that an analyzer will be used for only up to three samples per bioreactor per day. Off-line devices remain the primary reference analysis tool, but they present considerable limitations for use with real-time control strategies.

4. On-line Measurement Using Off-Gas Analyzers

Off-gas measurements are valuable for understanding the amount of CO_2 in the headspace of a bioreactor and can be used in conjunction with DCO_2 measurements to calculate parameters such as the Carbon Evolution Rate (CER).[3, 19] A major benefit of off-gas sensors is that they can be installed behind the sterile wall of a reactor, thereby avoiding any sterility requirements that in-line sensors face. They also do not suffer from the drift issues encountered with other types of CO_2 sensor technologies because they are based on InfraRed (IR) measurements rather than an indirect electrochemical method. The IR measurement enables the sensor to give additional data such as off-gas oxygen and the proportion of water as well. These benefits make off-gas CO_2 measurement a valuable addition to other CO_2 analytics.



Off-gas measurements provide an understanding of the average CO_2 level of the entire reactor. This differs from DCO_2 analyses that provide a measurement from a local environment (e.g. from where a sample was taken). Although they are measuring two different parameters, having an off-gas CO_2 measurement does provide a semi-redundancy for DCO_2 because the expected relationship between the phases can provide some insight. This expected relationship often leads to off-gas measurements being used as a proxy for dissolved CO_2,[9] but this should be done with caution. The presumed equality between these two measurement types is based on the assumption that the partial pressure of the liquid medium is in equilibrium with the gaseous headspace. The equilibrium can be calculated using Henry's law but is not necessarily an accurate representation of the real reactor. This is due to the many factors that can influence Henry's constant, a high potential for equilibrium to not become established during the course of a process, and desorption dependent on phase conditions. Generally, the partial pressure of CO_2 will be higher in the liquid than in the gas, otherwise desorption to the gas phase is not possible. This could lead to significant underestimation of DCO_2 if off-gas measurements are used in isolation.[10] Some literature shows that this can be acceptable only under specific limited conditions, such as low mass-transfer rates and non-steady state conditions[11] or cell-free bioprocesses.[12] Differences between DCO_2 and Off-gas CO_2 can become critical especially for large volume bioreactors where the surface area-to-volume ratio is much lower than in smaller reactors. Large disparities between the liquid and gas phases also occur in processes with limited or no antifoam. Foam forms at the interface between the liquid medium and the headspace resulting in a larger accumulation of CO_2 and other gases in the liquid than dictated by Henry's law. The retention of gases in the liquid phase prevents their detection by off-gas analyzers.

In general, off-gas analysis can be suitable for real-time dissolved CO_2 controls only under specific conditions, limiting its utility. The greatest risk is to underestimate the pCO_2 in the bioreactor. This risk should not be understated. Research shows that even brief excursions of CO_2 over the culture tolerance limits in the early stages of a process can have long-term detrimental effects, even when pCO_2 returns to reference values.[13] For this reason the in-line/in-situ measurement should be the analytical method of choice for DCO_2 control purposes when possible.

5. In-line Measurement

5.1 Electrochemical Sensors Adapted from pH Probes: The Severinghaus Principle

As described in the introduction and in the PAT guidelines, measurement of dissolved CO_2 in the bioreactor is most beneficial when a control system can be implemented to maintain the DCO_2 level to match the golden profile. Off-line methods have long been trusted to monitor CO_2 in the bioreactor medium but do not provide frequent enough data to be used in automated control systems Electrochemical sensors, like those developed for sensing dissolved oxygen, cannot be used for DCO_2 because CO_2 will not be reduced in water. Thus, in-line DCO_2 sensors for the bioreactor are based on the Severinghaus principle because it is suitable for use in liquid media and have been developed to meet the many requirements of biopharma applications.[14]

This technology was consolidated by Dr. John W. Severinghaus in 1957[16] and despite many efforts in research and development, the Severinghaus basic principle for potentiometric CO_2 sensors has remained almost unchanged since its introduction. For example, it is still the basis of most off-line BGAs, making the in-line data easily compared to a standard. Severinghaus-based sensors enable an in-situ, real-time measurement of CO_2 that can be used for gas or liquid measurements. These sensors use an indirect measurement method in which CO_2 crosses a CO_2-permeable membrane (commonly PTFE or Silicone) into an electrolyte solution (typically bicarbonate). Increased CO_2 concentration acidifies this solution. The resultant decrease in pH can be translated into a calculation of DCO_2 partial pressure. These calculations can be automatically performed in a complementary software, but the interactions of CO_2 and pH should be well understood before using the data.[17] This principle has been shown to be effective

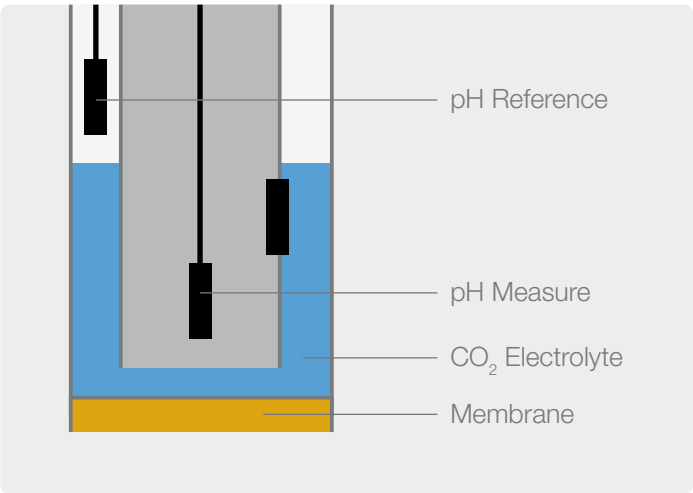


Figure 2: Main components and mode of operation of the Severinghaus carbon dioxide sensor. Exemplified from Carbon Dioxide Sensing Fundamentals.[15]

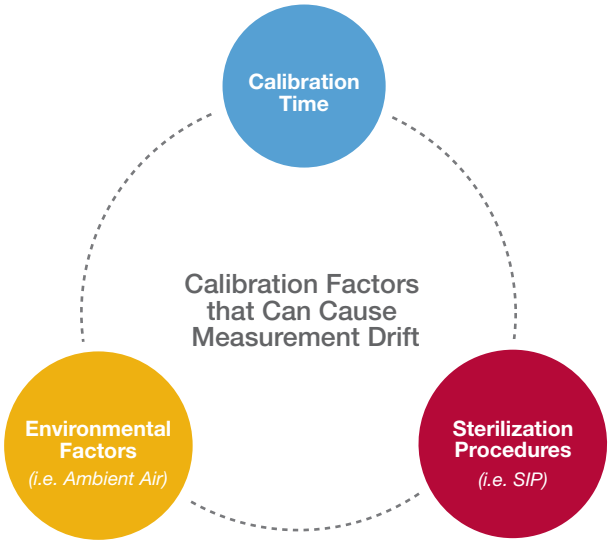
in typical bioprocesses but require significant handling and use considerations.

The most substantial hurdles in using a Severinghaus-based DCO_2 sensor are the maintenance and calibration aspects. The sensors are constructed from several different components working together that frequently need replacement or repair. The component most commonly in need of maintenance is the electrolyte solution. DCO_2 measurement may be affected by fouling or by electrolyte concentration, so the solution needs to be refilled frequently and replaced at each calibration. The membrane of these sensors must also be replaced before each calibration and should not be reused. For many users, this means replacing the membrane for each process, batch, or run. The most expensive and labor-intensive component to maintain and replace is the internal pH sensor. This component does not

need to be replaced as often as the membrane or electrolyte, but it is subject to the same difficulties as a typical pH sensor, especially when exposed to several autoclavation cycles. Additionally, the pH glass needs to be handled with extreme care. Contact with anything other than intended components (viz. electrolyte, buffers, or membrane) may cause irreparable damage, so skilled technicians need to ensure that they do not touch the glass with their hands or any other surface.

Calibration of Severinghaus-based sensors can be cumbersome and prone to error. One of the first steps of the calibration process is to remove the membrane body carefully without touching the pH glass. The internal pH sensor then needs to be calibrated similarly to a traditional pH sensor, although manufacturer recommendations include at least 10 minutes in a pH 7 buffer. This part of the calibration can also be used to determine whether the pH sensor is in need of replacement, for example, if the slope is out of specification. Once the pH sensor is calibrated, a new membrane body needs to be filled with electrolyte (air bubbles having been removed as much as possible) and carefully replaced over the pH glass. Finally, the sensor cap sleeve needs to be replaced, and the sensor can be installed. The calibration of electrochemical CO_2 sensors is particularly difficult if measurements under field conditions and in solutions of unknown and changing composition must be carried out. It was found that considerable deviations might occur between the analytically determined CO_2 concentrations and those measured with the sensor if the measuring and the calibrating solutions differ substantially in their composition.[15]

Even with a perfect calibration, there are several factors that need to be considered regarding measurement accuracy. Sterilization procedures, for example, can significantly impact the sensor reading, as mentioned. Autoclavation and other sterilization protocols (e.g. SIP) can impart an



offset in the measurement that needs to be corrected for with a product calibration step utilizing gas standards in the reactor. If the sensor is stored in ambient air for any period of time, a portion of the electrolyte may evaporate out of the membrane. In this case, the concentration will have changed, resulting in a drift in the measurement. The electrolyte solution would then need to be replaced. Longer processes may also experience significant drift due to instability in the electrolyte and membrane body. Volatile components and weak acids (such as the volatile formic acid) may interact with the electrolyte, reducing it and thereby changing the measurement (this is fairly uncommon as most mammalian processes do not reach problematically low pH levels). The many potential causes of drift require the sensors to be frequently recalibrated, even mid-process as is often required to retain in-spec measurements.

Last but not least, similarly to a pH sensor, Severinghaus-based sensors need to be installed at the proper angle in order to retain proper electrolyte contact, which is something to care for, especially in industrial-scale bioreactors.

5.2 Optochemical and Spectroscopic Sensors

In-line sensors have the inherent benefits associated with real-time measurement as urged by the PAT initiative, but at present, Severinghaus-based sensors are the only type truly suitable for measurement in the bioreactor. Fiber-optic probes can be used to connect to a large-scale spectrometer, but the high cost and complexity of this type of measurement preclude it from being a viable solution at the bioreactor in most cases.

An alternative was previously available in the form of a spectroscopy-based fiber optic (i.e. optochemical) probe-style sensors, but the option of this interesting technology is now quite difficult to find in the market. The discontinued sensors utilized fluorescence spectroscopy (in the UV-vis spectrum) to measure the concentration of CO_2 in the bioreactor. Removable capsules containing an electrolyte and dye (HPTS) solution could be inserted into a sensor body in order to integrate the sensor into the bioreactor. Like the Severinghaus-based sensors, the electrolyte in the capsule is acidified by the presence of CO_2. The dye contained in the solution has two fluorescent emission wavelengths. The ratio of the emission intensity of these two peaks vary with pH. Therefore, ratiometric analysis of the fluorescence emission can be used to determine the pH of the solution and, consequently, the concentration of DCO_2 in the medium.

In a notable study, Pattison et al. found this technology to have many of the sought after advantages of dissolved CO_2 sensors![18] As discussed in chapter 1, a suitable sensor should have an appropriate measuring range, response time, accuracy, and insensitivity to process changes. The response time of 6 minutes was determined suitable for the rate of change and need for adjustment typical of a mammalian culture. This time frame is certainly shorter than the process of sampling and analysis involved in BGA measurements and enables process control based on the in-line sensor data. The measurement range was specified at 0–180 mmHG CO_2, covering the typical working range of DCO_2 in mammalian cultures. This study also found that there was no significant drift or loss of accuracy with process duration (although different scales were affected differently), temperature changes, or changes to metabolite concentration. Comparisons were made between the optical dye technology and two BGAs. The offset between in-line and off-line measurements remained relatively constant. The BGAs were calibrated frequently with gas standards and were therefore assumed to have negligible drift. The difference between measurement types is to be expected based on sampling effects such as off-gassing. If the offset between in-line and off-line measurements is constant, the

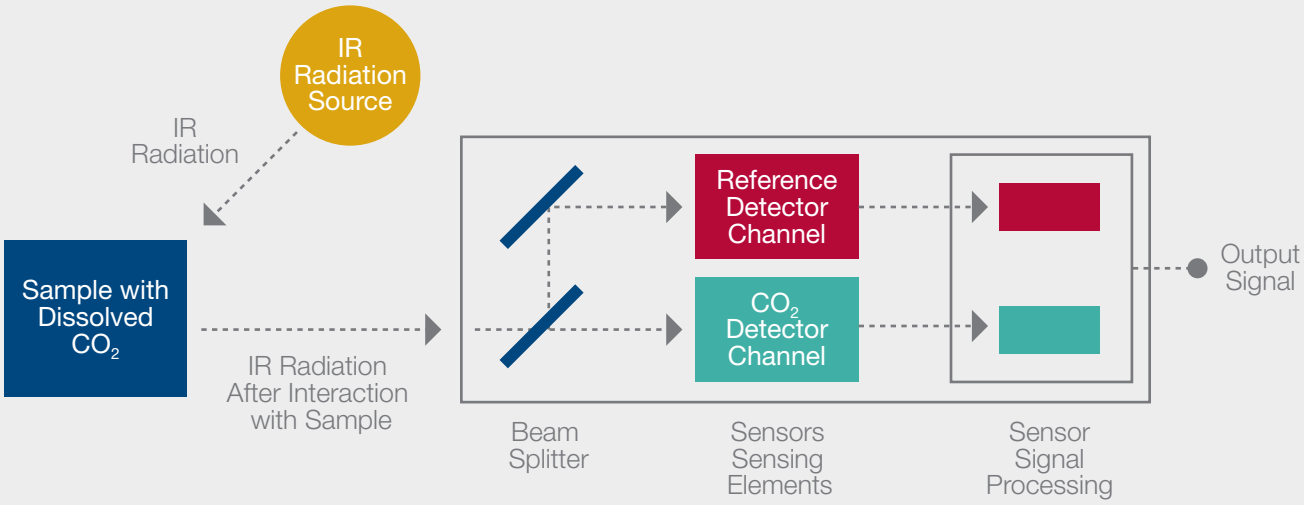


Figure 3: Generic scheme of main components and mode of operation of NDIR carbon dioxide sensor.

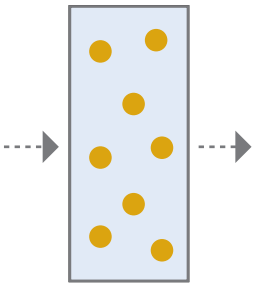
in-line measurement can be assumed to be a more accurate depiction of process conditions.

Other benefits of this type of sensor follow from the inherent benefits of ratiometric analyses including intrinsic protections against error from photobleaching, changes in source intensity, changes in pathlength, and other variations. This type of spectroscopic sensor combines the Severinghaus principle with optical techniques and thus carries the deficiencies of both types. Severinghaus-based sensors are discussed at length in section 5.1. Spectroscopic, or optochemical, sensors typically require significant data processing and a separate optical table to properly acquire and interpret a signal. This increased complexity may result in increased cost, points of failure, and required training to troubleshoot. This type of sensor has also been found to take up to 4 hours for calibration at the 30 L scale.[18]

The optochemical and mid-IR spectroscopic in-line sensors are based on exciting technology with many benefits towards bioprocessing. Along these lines, Nondispersive Infrared (NDIR) technology without the need for a dye shows a lot of promise. Sensors based on NDIR use the concentration-dependent absorption of electromagnetic radiation in the IR range, but unlike traditional spectrometers used to identify materials or gas mixtures, NDIR sensors do not comprise any dispersive optical component (Figure 3, page 11). This component is one of the most expensive elements of a spectrometer, so NDIR sensors are much less expensive than traditional spectrometers. This type of analysis utilizes a single transmitted wavelength that corresponds to the characteristic absorption band of the gas of interest (CO_2 in this case) rather than the entire spectrum. An increased CO_2 concentration absorbs more of the designated

Beer-Lambert Law

$$A = \varepsilon \ell c$$



A = Absorbance

ε = Molar Attentuation Coefficient or Absorptivity of the Attentuating Species

ℓ = Optical Path Length in cm

c = Concentration of the Attentuating Species

wavelength according to the Beer-Lambert law. This attenuation of wavelength can then be used to determine CO_2 concentration.

Such sensor technologies have the potential to perform accurate measurement of CO_2 in real-time. Additionally, they are almost always solid-state sensors and so can be mounted at any angle. Currently, there is an abundance of NDIR CO_2 sensors available, but their utility is aimed at measuring air quality, ventilation control in incubators or greenhouses, environmental and combustion control, and automotive applications, as well as for measuring dissolved CO_2 in freshwater.[20] The most difficult aspect of adapting this technology for use in the bioreactor is the elimination of water from the optical path. To the best of our knowledge, there is no in-line sensor capable of profiting from the advantages of NDIR, or optical technology in general, to measure dissolved CO_2 that is compatible with biopharma industry requirements such as miniaturization in 12 mm format (PG 13.5 process connection) and compliance with sterilization and cleaning procedures.

6. Conclusion

There are many ways to monitor dissolved CO_2 in the bioreactor at present and many factors to consider when choosing the most appropriate method for a given application (Table 1). For many applications, a combination of multiple methods will be the most beneficial. Soft sensors based on other measurements are complex and too high in effort for accurate and matrix-independent CO_2 analysis. Off-line measurements can be very useful for comparison against reference standards but do not provide frequent enough data for a true control strategy to be implemented. On-line off-gas measurements provide more continuous information but do not truly measure dissolved CO_2. To get the maximum process enhancement possible from CO_2, in-line measurement is required. Severinghaus-based sensors provide real-time continuous data on DCO_2, and at this time they are the only in-line option available. Unfortunately, they are high in maintenance needs and commonly held to be unacceptably low in accuracy and robustness for bioprocess control.[B] In-line IR optical sensors show potential in overcoming these deficiencies, but there are no options available in the form of reusable sensors suitable for biopharma applications.

Future improvements towards DCO_2 sensing should therefore be aimed at making an in-line sensor that is low maintenance, as well as accurate and robust, for process control. Such a sensor would also need to retain the characteristics required of biopharma sensors including a hygienic design and sterilizability. The ultimate goal is a solution which enables the proper control of CO_2 as a critical process parameter at the bioreactor.

Table 1. Evaluation of Benefits and Deficiencies of Most Common CO_2 Measurement Technologies

	Real-Time Monitoring	In-Situ Monitoring	Accurate Process Control Between 50–150 mbar	Robust Process Control *(Against SIP)*	Selective for Dissolved CO_2	Low Maintenance/ Low Effort	Independent Installation Angle
Soft Sensor	+	N/A	+	N/A	+	–	N/A
On-Line (Off-Gas)	+	N/A	+	N/A	+	+	+
Off-Line BGA Severinghaus	–	N/A	+ *(Low Range)* – *(High Range)*	N/A	–	++	N/A
Electrochemical Severinghaus	++	+	+ *(Low Range)* – *(High Range)*	+	–	–	–
Optical Severinghaus	++	+	+	+	–	+	+
In-Situ/In-Line Optical NDIR	++	N/A	++	++	++	++	++

Glossary

Definitions in this glossary have been derived from Hamilton White Papers[A, B] or the PAT guidelines unless otherwise cited.

Beer-Lambert Law

The Beer–Lambert law, also known as Beer's law, the Lambert–Beer law, or the Beer–Lambert–Bouguer law relates the attenuation of light to the properties of the material through which the light is travelling.[23] The law is commonly applied to chemical analysis measurements and used in understanding attenuation in physical optics, for photons, neutrons, or rarefied gases.

A common and practical expression of the Beer–Lambert law relates the optical attenuation of a physical material containing a single attenuating species (e.g. CO_2) of uniform concentration to the optical path length through the sample and absorptivity of the species. This expression is:

$$A = \varepsilon \ell c$$

A = Absorbance

ε = Molar Attentuation Coefficient or Absorptivity of the Attentuating Species

ℓ = Optical Path Length in cm

c = Concentration of the Attentuating Species

Carbon Evolution Rate (CER)

It corresponds to the CO_2 produced by the cell culture and/or microbial fermentation (mol/l h). The CER can be calculated subtracting the inorganic carbon pool development over time (corresponding to the $NaHCO_3$ evolution over time), from the CTR.[29]

CER = CTR – Inorganic Carbon Pool Over Time

Critical Process Parameter (CPP)

Critical Process Parameter according to the PAT nomenclature. It is a parameter whose variability has an impact on a critical quality attribute (CQA) and, therefore, should be monitored or controlled to ensure the process obtains the desired quality.

CPP Examples: pH, dissolved oxygen, and dissolved CO_2

Critical Quality Attribute (CQA)

Critical Quality Attribute according to the PAT nomenclature. It is physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality.

CQA Example: glycosylation patterns of monoclonal-antibodies. If the glycosylation patterns are not correct, then the proteins fold differently than expected, losing the therapeutic effect.

CTR - Carbon Transfer Rate

The carbon transfer rate corresponds to the transfer rate of CO_2 from gas to liquid phase in the bioreactor (mol/l h).

Dissolved CO_2

Dissolved CO_2 (or DCO_2) is a Critical Process Parameter in bioprocesses. A higher dissolved carbon dioxide level is toxic and can inhibit cell growth and reduce production of metabolites such as monoclonal antibodies (mAb). The dissolved CO_2 is commonly identified with the "Partial Pressure of CO_2" (pCO_2). The pCO_2 can be found expressed with different units in process analytics. To facilitate a comparison among suppliers and scientific literature a table with most common pCO_2 units conversion is presented.

pCO_2 Units	mbar	kPA	mmHg	%Vol
mbar	1	0.1	0.750	0.1
kPa	10	1	7.50	1
mmHg	1.33	0.133	1	0.13
%Vol	10	1	7.5	1

**At temperature = 25°C and Atmospheric Pressure P = 1,013 mbar*

Dissolved O_2

Dissolved Oxygen (DO or pO_2) is a bioprocess Critical Process Parameter. Air or oxygen-enriched air is supplied to the bioreactor to support cell demand. Oxygen is used for cellular respiration and cellular growth. While important, DO can be controlled over a broader range than pH without too significantly impacting cell growth rates or product quality. Typical DO operating ranges for aerobic cultures lie between 30 to 40% air saturation. DO levels below this range will affect cell viability, whereas excessive DO levels can oxidize the end-product.

US Food and Drug Administration (FDA)

The FDA is responsible for protecting the public health by ensuring the safety, efficacy, and security of human and veterinary drugs for the USA government.

Henry's Law

In aqueous solution at pH 7, dissolved CO_2 occurs mainly in two inorganic forms: free aqueous carbon dioxide (CO_2 (aq)) and bicarbonate ion (HCO_3-). The solubility of CO_2 in aqueous solutions is dependent on pCO_2 in the headspace as described by Henry's law.

$$H_{CO_2} = \frac{C_{CO_2L}}{P_{CO_2}} \left[\frac{\text{mmol}}{\text{L bar}} \right]$$

Henry's law helps to illustrate why DCO_2 is generally referred to in terms of partial pressure of CO_2 (pCO_2), as opposed to molecular concentration.[A]

K_LA

The K_LA corresponds to the volumetric mass transfer coefficient for oxygen or CO_2 of a bioreactor and it is one of the dimensions which characterize it.[6]

Key Performance Indicator (KPI)

Key Performance Indicator, according to the PAT nomenclature. A KPI is a metric for the status of each production step. KPIs are related to CQAs and therefore influenced, as well, by the CPPs. As the CPPs remain within the pre-defined limits, the KPIs should indicate that each production step proceeds accordingly resulting, in the end, in a product having its CQAs within the appropriate limits, too.

KPI Examples: viable cell density, culture viability, and product titer

Monitoring/Measuring At-Line

Measurement where the sample is removed, isolated from, and analyzed in close proximity to the process stream.

Monitoring/Measuring In-Line/In-Situ

Measurement where the sample is not removed from the process stream and can be invasive or noninvasive

Monitoring/Measuring On-Line

Measurement where the sample is diverted from the manufacturing process, and may be returned to the process stream.

Monitoring/Measuring Off-line

The sample is taken out of the bioreactor in sterile conditions and analyzed in the lab after physical pretreatments (e.g. filtration and dilution).

Oxygen Transfer Rate (OTR)

The oxygen transfer rate corresponds to the transfer rate of oxygen from gas to liquid phase in the bioreactor (mol/l h).

Oxygen Uptake Rate (OUR)

OUR corresponds to the oxygen consumed by the cell culture and/or microbial fermentation (mol/l h).

Process Analytical Technology (PAT)

The Process Analytical Technology guidance has been published in 2004 by the FDA. It is intended to describe a regulatory framework that will encourage the voluntary development and implementation of innovative pharmaceutical development, manufacturing, and quality assurance.

Respiratory Quotient (RQ)

Respiratory Quotient (RQ) is the moles of carbon dioxide evolved per mole of oxygen consumed by the culture during the bioprocess. It is an indirect but a fairly rapid method of measurement to determine the lack of substrate in the growth medium.[32] It is also express as:[28]

$$RQ = CER/OUR$$

Scale-Up/Scale-Down

Scale-up and scale-down are therms referring to the transfer of the bioprocess from the R&D stage to the pilot or production stage (scale-up) or vice-versa (scale-down). The production/pilot bioreactor design is often much different than typically found in the laboratory. The larger bioreactor volume resulting from scale-up slows down the OTR and impacts the CER, further resulting, for example, in slower DO detection. If the PID control algorithms are set for the smaller scale, the response will be inaccurate. The mass transfer coefficient for oxygen, K_LA, must be kept constant in the scale up process to accurately predict OTR or CER. Frequent test runs need to be performed to adjust the control algorithms for dissolved oxygen.

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