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Controlling Relative Humidity and Condensation in a CO_2 Incubator

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INTRODUCTION

A carbon dioxide (CO_2) incubator is the instrument that is typically chosen to provide optimal temperature, humidity and CO_2 conditions to grow and maintain microbiological or cell cultures. Technologies to provide precise, user-defined control of temperature and CO_2 are widely available, but methods to control relative humidity (RH) while preventing condensation are still evolving.

It is generally accepted that to create an environment conducive to culture growth, a controlled atmosphere with a temperature of 37°C, 5% CO_2 concentration and 95% RH must be created and maintained. For many applications, these parameter values are an accurate representation of the conditions in which cultures will grow with the expected characteristics and at the expected rates.

However, many microbes and specialized cell types have different requirements. For example, psychrotrophic and thermophilic bacteria grow best at temperatures below and above (respectively) 37°C (e.g., psychrotrophs *Vibrio marinus, Thiobacillus novellus*, and *Vibrio cholerae*; and thermophiles *Bacillus flavothermus* and *Thermus aquaticus*), making the manipulation of the temperature variable critical for the growth of such cultures. Additionally, when studying a stressed cellular system, the ability to manipulate relative humidity is advantageous.

For example, when studying the effects of *V. cholerae* on human gastrointestinal cells, the manipulation of all three environmental variables, including RH, is required, because the stress on the cellular system can be more accurately defined by mimicking the in vivo response to infection (i.e., dehydration).

Technologies to provide precise, user-defined control of temperature and CO_2 concentration are widely available, so these two variables are commonly manipulated for experimental purposes; however, the same is not true for RH, directly impacting research validity and reliability by negatively impacting culture growth and making it less predictable, especially when work is done in small volumes, like multiwell plates. Evaporation of the liquid growth medium will change the concentration of nutrients and waste products in the growth medium, leading to ineffective and irreproducible results.

Additionally, failure to carefully control humidification is a significant factor in the formation of condensate inside the incubator chamber, which encourages the growth and spread of contaminants and negatively impacts laboratory productivity and cleanliness.

This paper will explore the various factors that affect RH in CO_2 incubators, as well as evaluate the effects of the available humidity and condensation control.

DISCUSSION

Does Your CO_2 Incubator Provide Active Humidity Control?

In most incubators, relative humidity is not a parameter that is actively monitored and actively controlled. When assessing how suppliers represent their ability to control humidity, it is important to review the operating parameters and specifications they publish.

Before we examine the available technologies for humidity control and management, it is useful to define some terms.

- Active humidity control is the creation and maintenance of the relative humidity parameter around a user-defined set point.
- Active condensation control is the maintenance of surface temperature gradients above the dew point, such that condensate does not have an opportunity to form.
- Passive condensation management is diverting condensate that has already formed to an area away from incubating cultures.

Open Control Loops and the Illusion of Relative Humidity Control

Most CO_2 incubators deliver humidity to the chamber by directly or indirectly heating a pan of water to create water vapor. A microprocessor uses a mathematical formula to determine the required temperature of the heating elements to create a given humidity level. Some incubators provide just one humidity level without allowing the user to adjust it (i.e., there is no humidity "control"), while others allow the user to "dial in" to a specific humidity set point (e.g., 90%), which the system then attempts to maintain.

These latter types of systems give the user only the illusion of control. Real-world conditions vary significantly from mathematical ideals, and two important factors affect the actual level of humidity delivered. First, because CO_2 incubators are not sealed systems, ambient conditions affect the ability of an incubator to maintain set points. Ambient conditions vary from facility to facility, and a system needs to work harder to maintain a humidity set point in a cooler, drier laboratory than in a warmer, more humid environment. Second, everyday activities such as door openings also affect conditions inside the chamber.

Some laboratories are more active than others, and it will be more difficult to maintain humidity levels in those labs than in facilities with lower usage. In either case, without feedback about actual conditions in the chamber interior, it is not possible to know if the system is maintaining anywhere near the RH set point, let alone recovering in a reasonable amount of time following a day of multiple consecutive door openings.

Systems that deliver humidity in this way utilize an open control loop or non-feedback controller. This type of control does not collect or use feedback to determine if the system has reached the user-defined set point, and therefore, no corrective action is taken if it has not. It is a simple and relatively inexpensive technology, but the trade-off is that RH is unknown – a situation that could negatively impact cultures, laboratory productivity and, ultimately, costs. Some laboratories try to work around this weakness by monitoring RH manually with RH sensors purchased separately and, through a process of trial and error, experimenting to discover a set point that will work for their application. However, this approach is costly, and it is easy to incorrectly tune a desired set point when making manual adjustments. Additionally, it will not help mitigate the effects of lackluster humidity recovery following door openings in real-world conditions. And it will not help in a situation where an incubator is simply unable to generate the high levels of humidity its supplier purports to provide. Under normal daily operation (e.g., frequent door openings, variable ambient temperatures, etc.), a typical operating range is 85-90% RH at best, more often 60-70% RH, putting sensitive cell lines that are prone to desiccation at risk.

Simply operating the incubator at its highest humidity setting does not solve this problem, since it may take up to three hours to recover to just 90% RH following a door open or other event. Even when an incubator is able to reach 95% humidity, end users typically find significant condensate within the chamber upon opening the incubator doors.

True Active Humidity Control with a Closed Control Loop

To combat this, some incubator suppliers utilize an integrated RH sensor. This sensor is the hallmark of an incubator that provides true active humidity control. This type of incubator utilizes a closed loop controller that proactively samples the atmosphere inside the chamber and uses the data collected to determine if the set point has been achieved or maintained. If it has not, the system is programmed to make small, controlled adjustments as needed to create or maintain interior conditions at or near the set point with no significant overshoot.

A few suppliers have implemented such controls into their incubator models, but some place restrictions on the nominal set point that can be established. Others determine a specific operating range under which a user can control RH (e.g., ambient dependent to 90%, 0% up to 95%, etc.). The reasons for these restrictions tend to coalesce around an equally important reason to carefully control incubator humidification—condensation.

Solving the Condensation Conundrum

It is unfortunately accepted as given that CO_2 incubators experience condensation during normal operation. In fact, a close inspection of technical documents shows that incubator manufacturers themselves are aware of the problem, stating that operating at the highest humidity settings will almost assuredly result in condensation inside the chamber. Condensation is unavoidable in certain situations, such as when cooling the chamber interior. Because warmer air holds more water vapor, water condenses from the air as it cools. A common way to prevent condensation during cooling, if it is safe to do so, is to open the interior door to allow humidity to escape to the ambient environment. This type of unavoidable condensation is enough for laboratory personnel to manage without the increased burden of fogging interior glass doors and wet shelves/sidewalls during periods of normal operation.

The constant presence of condensate during normal operation is more than just minor inconvenience, as water clinging to the chamber interior provides appealing locations for contaminants to grow and spread. Additionally, significant exposure to condensate can cause the accuracy of capacitive RH sensors (the type of RH sensor used in most CO_2 incubators) to drift, and can lead to their failure, adding to increased maintenance costs, downtime, and the total cost of ownership of the incubator. Condensation is particularly an issue at higher levels of RH, causing a catch-22: dial down the humidity and risk dehydrating cells, or provide the high humidity your cells require and cope with wet surfaces. Further complicating matters is the fact that condensation removes moisture from the air, meaning relative humidity drops when condensation occurs.

Surface Temperature Gradient and Dew Point

Condensate forms when environmental parameters fluctuate (e.g., following door openings) and affect the surface temperature gradient inside the incubator chamber. The surface temperature gradient is a representation of the variance in temperature across the interior surfaces of the chamber. If any portion of the gradient falls at or below the dew point, condensate will form on the affected surfaces. Surfaces that typically fall below the dew point include areas that are along seams, such as corners, where heaters do not fit or reach.

Condensation can be prevented by using a number of technologies to keep surface temperatures above the dew point. Closed-loop controllers provide better control over air temperature and RH, both of which directly affect dew point. More stable temperature and RH parameters mean the dew point is more stable, reducing the chance that the dew point will drift into the surface temperature gradient. Additionally, the spatial arrangement of heating elements to evenly disperse heat throughout the chamber can help to minimize cold spots, narrowing the gradient and reducing the chance that a portion of it will extend below the dew point.

Some incubators can deliver a condensate-free environment and optimal RH levels, but not consistently, and to the point where recovery to the set point can be achieved quickly (within 6 minutes) and accurately, with minimal overshoot, maintaining the ideal growing environment of the cell cultures at all times. These factors vary considerably from model to model, introducing more unknowns and more need for trial-and-error to come up with a workable protocol. A strong need exists for a CO_2 incubator that provides active control of humidity while significantly reducing condensation, even at high humidity levels.

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Humidity and Condensation Control Technologies Overview

Active Control vs. Passive Management

Marketing claims about humidity control are muddled with confusion. First, it is not always clear whether a claim relates to controlling condensation or controlling the relative humidity parameter. Additionally, methods that claim to control condensation often do nothing to prevent it; rather, they simply manage the presence of condensate that has already formed by providing it with a place to collect somewhere inside the chamber. This is not active control, but passive management. And as previously discussed, many RH control technologies simply give the illusion of control, because of a lack of feedback from the system.

A close inspection of technical documents and specifications will show what a marketing claim is actually covering. A useful exercise is to arrange specifications side by side, to see if you are comparing apples to apples. **Figure 1** is an example of such a comparison.

FIGURE 1.

Humidity Management and Control Technologies Comparison

	INCUBATOR A	INCUBATOR B	INCUBATOR C	INCUBATOR D
Humidity Management / Control Technology	Passive Condensation Control only (no RH control)	Active Humidity Control / Limited Active Condensation Control	Active Humidity Control / Limited Active Condensation Control	Active Humidity Control / Limited Active Condensation Control
Humidity Control Range	Up to 95%	Ambient dependent to 90%	>90% @ 37°C w/active control	>90% @ 37°C
Humidification Source	Evaporative (Water Pan)	Evaporative (Water Pan)	Evaporative (Water Pan)	Evaporative (Water Pan)
Humidity Control Accuracy	± 5%	± 3%	± 2%	± 3%
Humidity Recovery	Within 3% of set point in 20-25 mins	To 90% +5%/-3% within 25 mins (average)	?	Follow a 30-second door opening, to 90% of initial value in 20-30 mins (time varies by published document)
Marketing Claim	"We can provide a high humidity environment without any unwanted condensation in the chamber and resulting contamination concerns."	"A unique blend of product features to promote cell growth through the precise control of humidity..."	"Full humidity control"	"Rapid Response Humidity System"
Evaluation	Claim applies to management of condensation only, not active humidity control. Condensation is passively managed through the use of a "dew stick" cooled to below the dew point by peltier cooling technology. However, this does not prevent the formation of condensation on other surfaces that are at or below the dew point.	Claim applies to active humidity control, with active condensation control available only up to 90% RH. Control accuracy is provided at start-up only. The accuracy after continued use varies widely, and recovery times are slow. It appears that the upper range of humidity controls is limited to 90% to prevent the formation of condensation at high humidity levels, where sensor reliability may be an issue.	Claim applies to active humidity control with active condensation control available only up to 90%. The claim to provide ">95% RH" comes with the following caveat: "Settings above 90% may cause some condensation to occur in the chamber."	Claim applies to active humidity control with active condensation control available only up to 90%. To achieve >90%, a switchover function allows the selection of two humidity settings (high & low). Active condensation control is available in the low humidity range; however, the elevated humidity range may cause condensation to form on the outer sides of the container.

It is easy to see how claims to manage or control humidity mean different things to different suppliers. Some do appear to use closed-loop feedback controllers to provide active control of RH. Others do not even provide the illusion of such control but seek only to passively manage condensation through clever gimmicks or mechanical devices aimed at reducing condensation within the system. Clear differences exist in methods used for humidification, mechanical design, sensors, and sensor accuracy.

The implication for condensation control is clear – technologies for preventing condensation are simply not available at humidity levels above 90%, turning incubators into ideal breeding grounds for contaminants, affecting laboratory productivity and adding to total cost of ownership when RH sensors fail.

Although suppliers of incubators are not being untruthful when representing the level of RH and condensation control they are able to provide, they are not telling you the whole story. In fact, the most pervasive claim about CO_2 incubators may be the least understood.

The 95% Myth

Most incubator manufacturers claim to deliver RH control of "up to 95%." While this may be technically true, the claim comes with a significant caveat related to the accuracy of capacitive RH sensors, the formation of condensate inside the chamber, and the frequency of actually achieving 95% RH.

RH sensor technology has improved over the years, becoming more economical, reliable, and accurate. But like any type of measurement instrument, all RH sensors are subject to some degree of inaccuracy. Furthermore, capacitive RH sensors are subject to an additional drift in accuracy over time, typically a certain percentage per year. The majority of incubator suppliers do not specify these variations in accuracy within the material they publish, simply stating that sensor accuracy is within a certain range at a particular level of RH. In a closed-loop system that relies on data feeding back from these sensors, accuracy plays a critical role in not only how well set points are maintained, but how high set points may be maintained.

RH Sensor Accuracy

When developing ReCO_2ver™, Baker researched the accuracy of the RH sensor and discovered that it varies depending on temperature, RH, and length of operation. Most incubator manufacturers simply publish one "best case" sensor accuracy, and do not disclose the variances or the conditions under which those variances occur. **Figure 2** shows the accuracy data for ReCO_2ver™'s RH sensor.

As is evident, the accuracy of the capacitive RH sensor not only drifts over time, but it will respond differently to different temperature and RH conditions. The sensor performs least accurately under the most commonly used temperature and RH set points (37°C and 95% RH) and when the sensor has been operating for more than 60 hours (also a common real-world occurrence). In fact, an incubator in its first year of use, in continuous operation at 37°C and 95% RH, may actually be delivering humidity levels as low as 88.0% (95% - 2.0% - 3% - 2%) and as high as 99.0% (95% + 2.0% + 2.0%).

Under these conditions, it is not possible to say with certainty that the system is maintaining anywhere near the 95% RH set point. All that can be said for certain is that at the upper level, condensate is likely to form in abundant quantities – a situation that Baker wanted to avoid when developing ReCO_2ver™. Based on the above test results, our engineers were able to isolate sensor accuracy as another variable that could be accounted for in order to virtually eliminate condensation inside the chamber, even at humidity levels above 90%.

Surface Temperature Gradient and Condensation Control

At high humidity levels, the surface temperature gradient continues to play a significant role in condensation inside the incubator chamber. Knowing this, Baker performed further analysis to determine the surface temperature gradient of ReCO_2ver™, showing that the gradient is comparable to that of competitor models: 35.5°C – 37.5°C, when at an air temperature set point of 37.0°C. Using the lowest surface temperature seen across the gradient (35.5°C) and the air temperature set point (37.0°C), the maximum RH that would not produce condensate was calculated.

FIGURE 2.
Accuracy Data for RH Sensor Used in ReCO_2ver™

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CONDITIONAL STATEMENT	RH SENSOR ACCURACY
RH is between 80-100% @ 25°C	± 2.0%
RH is greater than 80% for 60 hours	Additional + 3%
RH is between 0-70% from 15-55°C	Additional ± 1.5%
RH is between 70-100% from 15-55°C	Additional ± 1-2%
Yearly Drift	Additional <+ 0.5%

The result was 92%; that is, when RH is above 92%, the only condensate that will form will be on the surface areas of the incubator interior that have a temperature of 35.5°C (**Figure 3**). In $ReCO_2ver$™, these areas are small enough not to be noticed during normal use.

This is a significant improvement over competing models that can become saturated with condensate during normal use at RH set points over 90%.

Through this analysis, Baker has been able to determine the most optimal set point to use when high levels of humidity are required. Different from most incubator suppliers who claim "up to 95%" humidity control with caveats related to condensation, Baker has been able to establish an ideal range where greater than 90% RH can be delivered consistently while almost completely preventing condensation.

Not only does this provide an optimal setting for RH at high levels, but it protects the RH sensor itself, reducing downtime and ongoing maintenance costs.

Baker is proud to be the only supplier to study this relationship between sensor accuracy and surface temperature gradient.

We are also the only supplier to have accounted for that within a proprietary P.I.D. control algorithm (InteliCELL™) designed to create the most optimal environment for cell growth, where condensation is almost completely prevented, even while maintaining high levels of humidity within the system.

Tying It All Together – $ReCO_2ver$™ CO_2 Incubator

So far, this paper has discussed the importance of a closed-loop controller in maintaining RH to a user-defined set point, and how surface temperature gradient and sensor accuracy factor in to preventing condensation at high humidity set points. Baker's CO_2 incubator, $ReCO_2ver$™, has been designed with these factors in mind to provide true active humidity control and active condensation control. Through the use of InteliCELL™, a proprietary P.I.D. control algorithm (a type of closed loop controller), $ReCO_2ver$™ is designed to deliver not only active control over temperature and CO_2 concentration, but to deliver true active humidity control and prevent condensation.

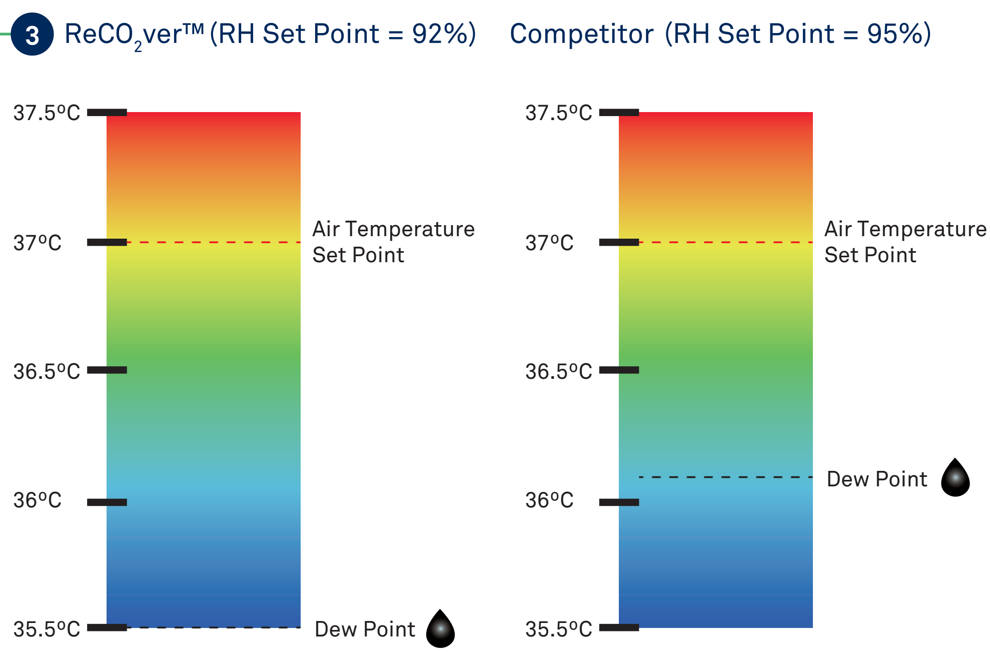

FIGURE 3.

Figure 3: Surface Temperature Gradient and Condensation at Two High Humidity Settings. Red (blue) represents the temperature of the warmest (coolest) surfaces inside the chamber. Condensation will occur on surfaces with temperatures at or below the dew point. Condensation inside $ReCO_2ver$™ is significantly minimized.

To do this, $ReCO_2ver$™ offers two humidity settings:

- Up to 90%
- 90% to 95%

At the lower humidity setting, users can dial in to any set point up to 90%. InteliCELL™ continuously samples RH within the chamber and makes adjustments as needed to maintain the user-defined set point. At the higher humidity setting, InteliCELL™ uses the optimal set point that Baker defined in developing $ReCO_2ver$™ – 92% RH. InteliCELL™ factors in the accuracy and stability of the RH sensor and the surface temperature gradient in its control algorithm, so users should experience little to no condensation when operating at this higher level of humidity – a feature that no other CO_2 incubator supplier currently provides.

All specifications related to humidity control in the $ReCO_2ver$™ are provided in **Figure 4.**

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FIGURE 4.

Accuracy Data for RH Sensor Used in $ReCO_2ver$™

MATERIALS	$ReCO_2ver$™
Humidity Management / Control Technology	Active Humidity Control / Active Condensation Control
Humidity Control Range	Up to 90%; 90-95% (92% set point)
Humidification Source	Ultrasonic (Nebulizer)
HUMIDITY CONTROL ACCURACY	
RH is between 80-100% @ 25°C	± 2.0%
RH is greater than 80% for 60 hours	Additional + 3%
RH is between 0-70% from 15-55°C	Additional ± 1.5%
RH is between 70-100% from 15-55°C	Additional ± 1-%
Yearly Drift	Additional <+ 0.5%
Humidity Recovery	Within 3% of setpoint in 4 minutes

CONCLUSIONS

The market is saturated with CO_2 incubators that claim to provide "optimal" conditions for cell growth. But the definition of "optimal" is not always clear, and not always optimal for the end user's work, providing consistent growing conditions with fast recovery rates. End users need to arm themselves with information about whether their incubator provides true active humidity control, only the illusion of control, or no control at all. Considering an incubator such as ReCO_2ver™, which allows users to control RH in addition to temperature and CO_2, can help provide an environment that is truly optimal for a wide variety of cells, cell lines and applications.

Users also need to know when they should expect condensation to occur within their current incubator and what technologies it uses to either prevent condensation altogether or control it when it forms.

Reading the fine print in not only marketing literature, but also in technical documents such as product specifications and user manuals, will shed light on the particular technologies utilized in each system. An incubator like ReCO_2ver™ that factors in surface temperature gradient and RH sensor accuracy in its control algorithm can help reduce condensation concerns while providing unparalleled, user-defined control over another variable critical to all research applications: RH.

To provide the optimal environment for cells and cell growth, choose a solution that improves experimental control, enhances the repeatability of your work, and provides precise control over each of the three variables critical for achieving excellence in your research—temperature, carbon dioxide, and relative humidity.