# Recommendations for $CO_2$ Enrichment
## in Plant Growth and Mammalian Cell Culture Research

This resource article explains the benefits of $CO_2$ enrichment for two major controlled-environment research applications: plant growth and mammalian cell culture. While the biological mechanisms differ between plants and mammalian cells, $CO_2$ enrichment is integral in both research applications for achieving reproducible, high-quality experiment results. Within this resource, Percival Scientific also offers guidelines for use of additive $CO_2$ within these research applications. These guidelines are intended to serve as general recommendations and should be considered as a starting point rather than a definitive standard.

### Role of $CO_2$ In Natural Plant Growth

$CO_2$ makes up about .040-.045% of the natural atmosphere, which is roughly 400-450 parts per million (ppm). Plants absorb $CO_2$ through the pores in their leaves (stomata) as a key component of photosynthesis, the chemical process by which they make sugar (glucose) for energy and growth, releasing oxygen as a byproduct. They oxidize the stored sugars through respiration, producing $CO_2$ as a byproduct. In the natural environment, $CO_2$ levels fluctuate but stay within a vital balance for plants to survive and grow.

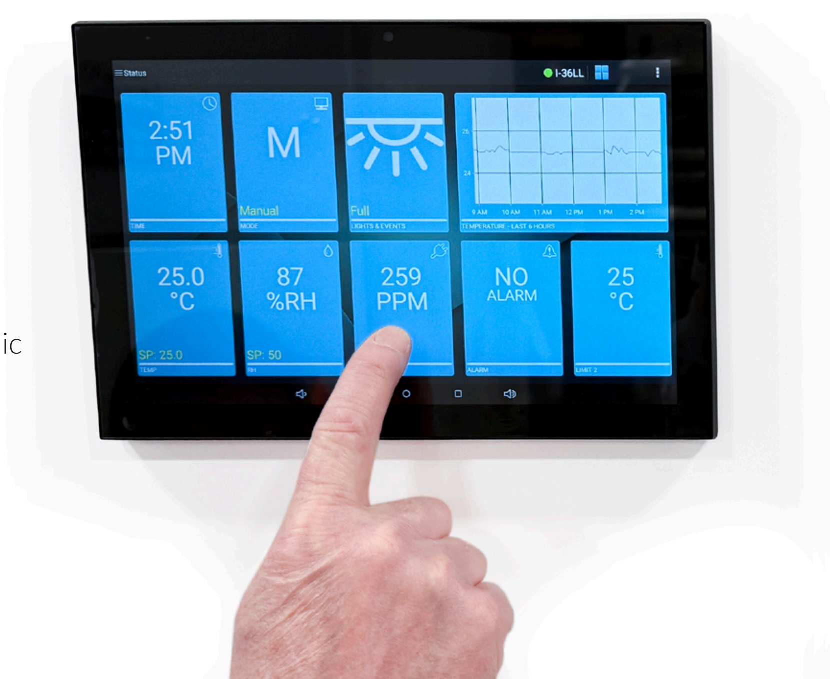
### Impact of $CO_2$ Enrichment Within Controlled Environments

In a sealed, controlled environment, plants may become deprived of $CO_2$ because the amount used during photosynthesis consistently exceeds what is produced during respiration without natural airflow. A low $CO_2$ level (or $CO_2$ stress) decreases the rate of photosynthesis, which subsequently lowers growth rates and overall plant mass.

Adding a $CO_2$ enrichment and removal system to a controlled environment not only prevents $CO_2$ levels from falling too low or rising too high, but it can also significantly boost photosynthesis (growth) rates and yields when the ambient level is raised to 700-1000 ppm. This has a large impact on plant propagation in many species. Other benefits of increased $CO_2$ levels include:

- Shorter crop cycles (faster development to maturity)
- Larger root systems, blooms and fruits
- Increased biomass
- Reduced plant transpiration and water intake

Raising ambient $CO_2$ levels to 1000 ppm is generally considered beneficial for many plants, and some species, especially hydroponic plants, can see benefit up to 1800 ppm; however, the advantage is usually minimal past 1500 ppm.



## Factors to Consider When Using $CO_2$ Enrichment for Controlled Plant Growth

### Plant Type

Levels of ambient $CO_2$ that exceed a plant type's needs can cause damage, so it's important to know the ideal $CO_2$ range for the plants you are growing. Certain plant varieties, such as geraniums and aster lilies, are more responsive to additive $CO_2$, while others, such as grasses, may show more modest responses. You should consider that the cost and effort of monitoring and keeping other growing parameters in balance with higher $CO_2$ levels may outweigh the yield benefits for some plant varieties.

### Adjustment of Other Growing Parameters

Adding $CO_2$ to a controlled environment can create an imbalance with other growing parameters, requiring adjustments for optimal growing conditions.

Light – An elevated $CO_2$ level requires a corresponding increase in light intensity for a higher rate of photosynthesis. However, increasing light intensity beyond the plant's light saturation point can contribute to causing tip burn and other damage, so these growth factors must be carefully balanced with this in mind. As light intensity is raised, $CO_2$ levels can be gradually increased.

Irrigation – Plants transpire less as $CO_2$ levels increase, so less irrigation is needed. The moisture content of the growing medium should be monitored to prevent oversaturation, and hydroponic plants will need less frequent water replenishment.

Temperature and Humidity – Adding $CO_2$ to the environment raises plants' temperature and humidity requirements for optimal photosynthesis. Refer to a vapor pressure deficit (VPD) guide to identify the optimal ranges for your environment.

Nutrients – Because higher $CO_2$ levels increase plant growth rate, resulting in larger root systems and more fruiting, plants consume nutrients more quickly and need more fertilizer. The mixture and ratio of minerals in the fertilizer may also need adjustment, as minerals like calcium, iron, zinc and boron have been shown to deplete faster than others.

Air Speed – Good air circulation is essential for evenly distributing $CO_2$ throughout the controlled environment, but airflow should not exceed 0.7 m/s and ideally remain below 0.3 m/s to prevent irreversible damage to plants. See *The Detrimental Impact of High-Speed Airflow in CEA* for a detailed report on air speed recommendations.

## Recommendations for Using $CO_2$ Enrichment in Controlled Plant Growth

- Know the optimal growth parameter ranges for the selected plant species and monitor them closely to prevent exceeding or dropping below the maximum or minimum limits for $CO_2$, light intensity, temperature, humidity and other factors.
- Use with early-stage plants for optimal growth rate results.
- Use $CO_2$ in its pure form; if this isn't possible (e.g., when using a generator), provide sufficient oxygen for combustion to prevent the formation of toxic gases.
- Ensure $CO_2$ is distributed above the plants with proper airflow that stays below 0.3 m/s.
- Do not add $CO_2$ directly to the nutrient solution of hydroponic plants, as this can lower pH levels; enrichment should occur only in the air.
- IMPORTANT: $CO_2$ levels above 5000 ppm can begin to be harmful to people and can be harmful to plants at or above 2000 ppm. Carbon monoxide should not exceed 50 ppm. Monitor levels carefully with sensors and set alarms appropriately. Percival recommends calibrating sensors monthly.

## Impact of $CO_2$ Enrichment in Cell Tissue Culture Research for Biological, Medical and Pharmaceutical Applications

In cell tissue culture research, $CO_2$ in the air of a controlled environment helps maintain the vital pH balance of the synthetic bicarbonate-buffered cell growth medium, which mimics the physiological state of the human body. Incubators and tissue culture chambers that combine a $CO_2$ enrichment and removal system with HEPA filtration to ensure sterility can create the ideal environment of 5% $CO_2$ in the air and a pH of 7.4 in the growth medium.

A carefully regulated balance of $CO_2$ in the environment enhances cellular viability and health, allowing cells to reproduce and grow. This significantly advances cell culture research, including drug testing and screening, vaccine development, stem cell research, genetic engineering, toxicology, disease modeling and regenerative medicine. The importance of $CO_2$ control in mammalian cell culture research is gaining more attention and study, becoming a key element for obtaining accurate experiment results and making impactful discoveries in human health and medicine.

## Balancing Other Cell Culture Research Parameters With $CO_2$ Enrichment

Along with a $CO_2$ concentration of 5-10% in the air and a growth media pH of 7.0-7.7, depending on the cell strain, other research parameters must be tightly controlled and monitored to mimic the sterile physiology of the human body as closely as possible.

**Temperature** – The environment must be kept at a stable 37°C for optimal enzyme activity and metabolism in human cells. For pathology studies, the temperature can be easily adjusted within a controlled environment to simulate the effects of illness or disease.

**Humidity** – Maintaining humidity around 95% with distilled or deionized water minimizes evaporation of the cell growth medium, which is vital for cell health. At the same time, chamber design and airflow must control condensation to preserve sterility. See *Factors Affecting Petri Dish Condensation in Tissue Culture Chambers*.

**Air Filtration** – A HEPA filter removes airborne particulates and microbial contaminants, reducing the risk of contamination from bacterial and viral aerosols. The use of HEPA air filtration is essential for maintaining a clean research environment and ensuring the most accurate experiment results.

## Recommendations for Using $CO_2$ Incubators and Tissue Culture Chambers

- Use pure, industrial-grade $CO_2$ to avoid contaminants.
- Monitor $CO_2$, temperature and humidity levels closely with sensors, and set alarms to activate when levels are out of the preferred range. Calibrate the sensors monthly.
- Follow the chamber manufacturer's protocols for using the $CO_2$ enrichment and removal system.

## We Offer Customizable $CO_2$ Products Right for Your Research

Percival Scientific provides a variety of $CO_2$ enrichment and removal systems to fit your preferred sensor and control ranges. Our plant growth chambers, walk-in rooms, $CO_2$ incubators and tissue culture chambers are known for their long-lasting, durable construction and precise control for consistent performance. Our IntellusUltra control system adapts to nearly any programming style and features a port for accessing SCADA/LIMS to customize data recording and tracking, ensuring compliance with regulations such as CFR21 Part 11 or GMP Annex 11. If you don't see what you're looking for in our product catalog, just give us a call at **800.695.2743** for a free consultation about how we can customize our products to suit your unique research needs.