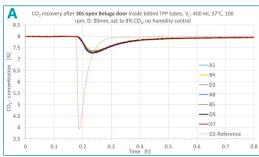
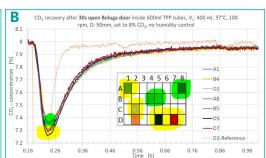
# CO<sub>2</sub> stability inside the vessel What happens when the Beluga door opens?

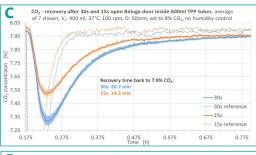


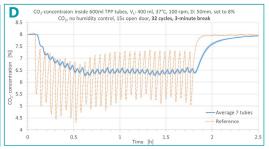


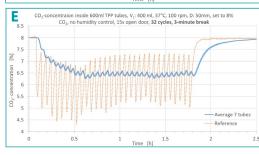
ISF1-Z Basic Beluga Perfect fit for automation

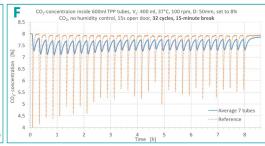












# Why this matters

Researchers often worry that frequent door openings in  $CO_2$ -controlled incubator shakers will disturb pH-relevant  $CO_2$  levels inside their cultures. While chamber  $CO_2$  can change rapidly, the key question is: **What happens inside the cultivation vessel itself?** 

### Measurement approach

Using the **Kuhner TOM** off-gas analysis system, CO<sub>2</sub> was measured directly in the headspace of **600 mL TPP TubeSpin® bioreactors** during shaking via a bypass—without vessel-internal sensors or wires.

## What actually happens when the door opens

Opening the Beluga door for 30 seconds cut the chamber  $CO_2$  from 8% to ~4%. Inside the tubes, however,  $CO_2$  decreased only **~10%**, stabilizing around 7.3%  $CO_2$ . The response was **slow, modest, and nearly identical** across all vessel positions (B). Shorter openings (15 s) caused even smaller changes (C).

#### Repeated door openings

Frequent openings led to a **stable oscillating equilibrium** inside the tubes (D+E+F)

- 3 minute breaks  $\rightarrow$  ~6.4% CO<sub>2</sub>
- 8 minute breaks  $\rightarrow \sim 7.3\% \text{ CO}_2$
- 15 minute breaks  $\rightarrow \sim 7.5\% \text{ CO}_2$

Despite heavy disturbance of the chamber atmosphere, **in-vessel conditions remained stable and close to the setpoint**.

#### Take-home message

- The CO<sub>2</sub> inside shaken vessels is remarkably **robust** against door openings.
- Even large chamber fluctuations cause only **small, slow changes** inside the tubes.
- Repeated openings produce a predictable equilibrium In this study between 0.5–1.6% below the setpoint.
- This effect is highly **reproducible**, and with an appropriately higher  $CO_2$  setpoint (via software control), the **deviation can be eliminated**.

