**Alm Lab Anaerobic Chamber Protocols**

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**Chamber Components:**

Environmental gas mixture – 5% Hydrogen, 20% Carbon dioxide, balanced with Nitrogen (AirGas, custom gas so no catalog number). This mixture takes ~3weeks to come in so there should always be a backup tank stocked in the lab.

Gas monitor (CAM-12) – monitors Oxygen (PPM) and hydrogen (%) in chamber, if change balance of hydrogen, nitrogen, or Carbondioxide in gas mixture used in chamber please see the CAM-12 user manual and contact Andy to schedule him to re-calibrate the monitor

Catalyst tower – scrubs air of oxygen in the presence of hydrogen. Needs to be baked in the incubator at 300 degrees for 3 hours every week. This unit also heats and displays the temperature in the chamber. To change the temperature in the chamber hit “set” button, adjust using the arrow buttons, then press enter. Number displayed it current temp unless press set.

Yellow lever on gas respirator – this controls the flow of gas from the tanks. If the lever is perpendicular to the gas line the valve is close, if parallel the valve is open and flowing into the chamber.

Gas regulator – gold rod = pressure controller for tank, monitor on left = pressure gauge of flow from tank, monitor on right = amount of gas in tank

**General Use:**

* If chamber has not been used recently it will need to be initialized.
* Chamber will need to be gassed with environmental gas mixture every other day when in use
* Pass through needs to be gassed with nitrogen before opening the inside door
* When passing materials in and out of the pass through please make sure you have gassed the chamber and the hydrogen is at least 2%
* If you are doing many pass throughs change the catalyst every 4 days

**Initializing the chamber:**

Note: This chamber uses a lot of gas and needs a lot of attention so when it is not in use it will not be maintained. So if it hasn’t been used in a while you will need to go through the following steps.

* All electronics should remain on even when the chamber is not in use.
* From inside the chamber, open the inner pass through door.
* On the nitrogen tank (black) regulator, turn the yellow lever parallel to the tubing to open valve and open gas flow through pass-through and into main chamber.
* VERY GENTLY AND SLOWLY twist the gold bar on the face of the regulator to the right to increase the pressure flow from 5psi to 15 psi.
* Allow the chamber to be gassed by Nitrogen for 20 minutes at 15psi.
* VERY GENTLY AND SLOWLY twist the gold bar on the face of the regulator to return the pressure flow to 5psi.
* Turn off the gas flow from the nitrogen tank by turning the yellow lever on the regulator perpendicular to the tubing to.
* Pass in a freshly baked catalyst (see below) and place it into the tower.
* On the environmental tank (green) regulator, turn the yellow lever parallel to the tubing to open valve and open gas flow through pass-through and into main chamber.
* Allow environmental gas to flow into the chamber for 5 minutes.
* Give the CAM-12 ~10 minutes to adjust before trusting the reading it gives.
* If you are concerned about the state of the chamber the level of oxygen can be tested using resazurin strips (stored on the very bottom shelf of the tall 4C)

**Gassing Chamber/Pass-through:**

* Chamber door to pass-through should remain closed when not in active use
* Any time you wish to put something into the chamber place it in the pass-through and de-gas the pass-through before opening the inner door to the chamber
* De-gassing the pass-through (Nitrogen):
  + Double check door into main chamber and confirm is closed before opening outer pass-through door
  + Place material in pass-through and close outer door
  + On the Nitrogen tank regulator, turn the yellow lever parallel to open valve and open gas flow to pass-through
  + After 60 seconds turn yellow lever perpendicular again to close the gas flow to the pass-through
  + Using the gloves in the main chamber, reach in and open the inner door to bring materials into main chamber
* De-gassing the main chamber (Environmental gas mix, hydrogen levels below 3%):
  + On the environmental gas regulator, turn the yellow lever parallel to open valve and open gas flow through pass-through and into main chamber
  + Continue until hydrogen levels have reached ~4%
  + Turn yellow lever perpendicular again to close the gas flow to the pass-through and chamber

**Refreshing catalyst in catalyst tower:**

* Catalyst trays should be baked every once a week
* Remove trays and bake them for 3 hours in the Voigt lab oven at setting 4
* The oven is located in the basement of NE47 in room 015
  + The code to this room is 4+5-1

**Calibrating the CAM-12:**

NOTE: only one person in the lab should be doing this to help minimize confusion

* The user guide for the CAM-12 is stored in the top drawer underneath the large vinyl chamber.
* To calibrate the monitor to ambient see page 10
* To set the CO2 levels on the monitor see pages 10-16

**Changing out gas canisters:**

NOTE: only one person in the lab should be doing this to help minimize confusion and risk

* NEVER move gas canisters without the safety controller cap
* On empty gas canister: Loosen pressure on respirator by twisting golden rod to the left.
* Use wrench to remove regulator from tank and place controller cap back on
* Move empty gas canister to wall bracket and exchange for full canister
* Once canister is in place and buckled down, twist off controller cap
* Cut off plastic wrap
* Screw on regulator
  + Make sure pressure controller (gold rod) is loose
  + NOTE: regulator is reverse threaded so Left = tighty and Right = loosy
* Turn knob on tank to open flow of gas to regulator
* Tighten tank pressure controller (gold rod) a little until pressure from tank is about 15 psi (will only be accurate if gas is actually flowing to chamber)