ENVIRONMENTAL HEALTH AND SAFETY
University of Nebraska-Lincoln

Safe Operating Procedure

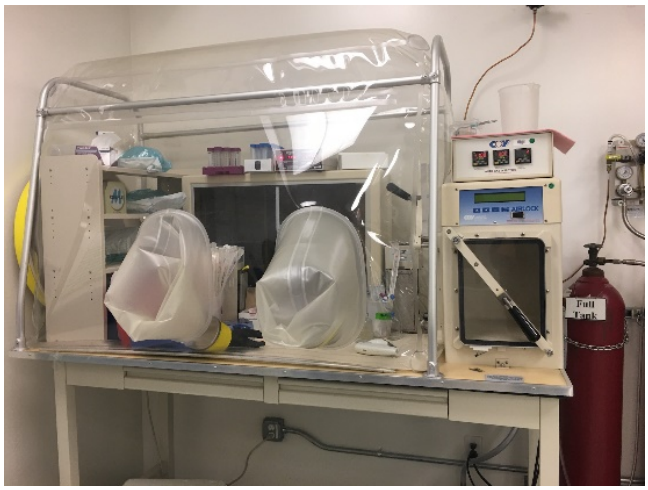
(Created 11/21)

ANAEROBIC CHAMBERS

Introduction and Scope

Anaerobic chambers are designed to provide an oxygen-free environment to facilitate growth of anaerobic microbes. The anaerobic chamber is purged with an inert gas, generally nitrogen, to remove most oxygen. The inert gas is then replaced with the desired gas mixture, typically 95% nitrogen/5% hydrogen. In combination with a catalyst, such as palladium, the hydrogen reacts with residual oxygen to fully achieve an anaerobic environment. An airlock is used to minimize introduction of air into the chamber while transferring samples into and out of the chamber.

Depending on user needs, anaerobic chambers can be found in many configurations, sizes, and with various gases (e.g., Argon chamber). Examples of these chambers are seen here:



Hydrogen Gas Safety

Hydrogen has a wide flammable range. A fire or explosion can occur when hydrogen gas at a concentration within its flammable range comes in contact with an ignition source. It is critical that the concentration of hydrogen inside the chamber maintained at or **below** 5% at all times. Users are advised to order from their gas supplier pre-mixed gases with a hydrogen content of no more than 5%.

To reduce the risk of fire or explosion, please consider the following:

- Prior to introducing the hydrogen gas mixture, purge chambers, airlocks, and antechambers with 100% nitrogen (or another inert gas as advised by the chamber manufacturer).
- Verify that the correct hydrogen mixture was shipped by the gas supplier *before* connecting new cylinders.
- Use an oxygen/hydrogen gas analyzer to monitor gas levels inside the chamber. The monitor will detect a problem with the gas mixture, valves or regulators.
- Leak test gas lines and the anaerobic chamber ***before*** start up.
- Never use a malfunctioning chamber. Use "**Out of Service**" signage if needed (see decommissioning section for details on signage).

Setup/Commissioning

- As with any laboratory equipment, proper setup is crucial. Please consider having a company representative onsite to ensure proper assembly, gas flow, and calibration of any alarms or sensors.
- Always read and adhere to the manufacturer's set-up and operating instructions.
- Do not modify the chamber from the manufacturer's original design.
- Contact EHS with any questions or concerns about placement or operation of the chamber.

Biosafety

Anaerobic chambers are often used for manipulation of anaerobic microorganisms, such as *Clostridia spp*. or methanogenic bacteria.

Note: Any work with human, animal, or plant pathogens, or transgenic organisms must be registered with and approved by the IBC prior to initiation of experiments (ibc@unl.edu).

While the anaerobic chamber provides a controlled atmosphere within containment, it **is not** identical in purpose or function to a biosafety cabinet. It should never be assumed that presence of oxygen is toxic to the microbes being manipulated as they may be categorized as either aero-tolerant or facultative anaerobes.

Material should never be removed from an anaerobic chamber without conducting surface disinfection with an appropriate and approved disinfectant.

Follow the guidance outlined in the EHS SOP, ***Microbiological Laboratory Practices*** when working with any type of microorganisms.

Decommissioning

If the chamber is to be removed from service, either temporarily (malfunction, repairs, etc.) or permanently (disposal), consider the following:

- Ensure all materials and equipment have been properly removed from chamber. Clean any spills.
- Ensure all compressed gas has been turned off and disconnected. Remove regulators and replace valve caps on compressed gas cylinders. Contact the gas supplier for cylinder removal if necessary.
- Contact the Facilities Service Desk (servicedesk@unl.edu or 402.472.1550) for gas decontamination of the chamber if experiments involved microorganisms.
- Place "**Out of Service**" signage on cabinet to prevent unauthorized use.
    - Signage should, at a minimum, include the words "**OUT OF SERVICE,**" the date taken out of service, and contact information for the custodian of the device.
- Notify UNL Inventory, Asset, and Surplus Management (unl_Inventory@unl.edu) if the chamber is to be declared surplus, moved to another institution, or assigned to a new user/department.