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# Anaerobic (vinyl) tent maintenance

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Works for me

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SoWa RI Anaerobic and Molecular Microbiology (public)

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## ABSTRACT

The [Coy vinyl anaerobic chambre](#) is a special device that is constructed by a purpose to create and maintain anaerobic environment. It is made from flexible PVC vinyl supported by tubular aluminum frame. The chambre is a totally sealed unit except two entry ports: the equipment entry port and the airlock.

**Airlock** is used to pass items between the chambre and the outside environment without disrupting the chamber's anaerobic atmosphere. For this purpose an automatic program (P1) consisting of three evacuation and three filling steps (two with N<sub>2</sub> and last one with gas mix) was established.

Remember:

- A) Light objects like towels will be flying or slightly moving in an Airlock during the procedure because of an over- and down-pressure changes. Be careful about a staff you are putting in, protect it from a damage.
- B) Everything that is tightly closed (bottles with media or chemicals, tubes etc.) has to be made anoxic before putting inside. Also consumables that are pre-packed (sterile serological pipettes, tips, Petri dishes) will still keep some oxygen after the Air-lock procedure. In some cases it is useful to make a small holes into packaging before to improve speed of gases exchange but it will lose the sterility.

Lab personnel access the chambre to perform their operations through **glove ports**. It is in favour of hygiene to wear one extra single-use gloves (nitrile, cotton or any others) under.

Anaerobic atmosphere inside the tent is created usually by nitrogen as inert background gas (aka **purge gas**) and by the **hydrogen gas mix** depending on the experimental demands (usually mix of N<sub>2</sub>, H<sub>2</sub> and if needed CO<sub>2</sub>). However, the hydrogen concentration inside the tent must be always kept under 5% unless the gas mixture may be explosive. 4% of hydrogen is an optimal concentration that is non-flammable and still provides enough hydrogen for oxygen removal. In our anaerobic chambre gas mixture N<sub>2</sub>/H<sub>2</sub> (95%/5% respectively) is used.

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## CREATED

Jun 22, 2018

## LAST MODIFIED

Sep 09, 2020

## PROTOCOL INTEGRATED IN

## GUIDELINES

- **Maintaining an anaerobic environment**

1. Gas Supply Tanks

- be sure there is enough gas in the bottles (check the pressure). For chamber operating 20 psi (138 kPa) is recommended. Because of the distance of gas bottles to chamber the pressure on regulator should be adjusted

- to approx. 40 psi (276 kPa).

- when a supply tank is empty replace it with full one (Messer Technogas company, České Budějovice store). Make a note into a log book.

2. Gas line leaks

- If there is a suspicion of gas leakage, prepare water with soap and spread it on the inspected surface. Gas leaks if there is a bubble formation.

3. Catalyst Stak-Paks (metal grid with **black** balls inside)

- rejuvenate catalyst periodically: bake Stak-Paks in an oven at 125 - 200°C for 2 hours once a week

- replace the catalyst once a year because it loses its effectiveness

- in order to test catalyst effectiveness place the pack into Air-lock, put a thermometer on it, manually evacuate inside atmosphere, fill the chamber with gas mix, wait for 10 min. Stack-Pak is supposed to be hot as the catalytic reaction is running. Temperature of Stak-Pak should rise up by 10°C if working properly.

4. CAM 12 (Anaerobic monitor H<sub>2</sub>/O<sub>2</sub> conc.)

- calibrate sensors once a year: remove sensors from behind and send to company (Accela s.r.o., Služeb 4, Praha 10, 108 00, CZ) for calibration.

- **Controlling moisture**

1. Dessicant Stak-Paks (metal grid with **white** balls inside)

- need to be rejuvenated periodically for 2 hours at 125 - 200°C.

2. Large Capacity Dehumidifier

- let the accumulated water come out from the tube (there is a container for this)

- **Hydrogen Sulfide control**

- Hydrogen Sulfide Removal Column requires no maintenance, except for replacing the media/catalyst every several months (indicator tells you -> becoming grey/blackish).

- **Vacuum pump**

- draining the moisture trap when full

- **Airlock**

- check sealings for leaks

- grease the airlock door push pins

- clean the seals to remove dust, dirt, and grime. Use water and soft cloth, do not use alcohol! You can also use

- wet sponge soaked with a little of soap or mild detergent. Allow the sealings to get dry before close the door.

- **Cleaning the vinyl**

- non-abrasive plastic cleaner and a soft cotton cloth, do not use paper towels that can scratch the vinyl.

- to disinfect the chamber use isopropylalcohol or a 1% to 5% chlorine bleach solution, **wipe completely** otherwise

it will **yellow** the vinyl.  
- **avoid** products containing **ketons** (for example acetone) it will damage PVC !

#### MATERIALS

NAME	CATALOG #	VENDOR
Vinyl Anaerobic Chamber	7200220	
Catalyst refill (180g)		

#### SAFETY WARNINGS

Gas mixtures containing more than 5% of hydrogen may be explosive!

#### BEFORE STARTING

Prepare and put inside the tent all the stuff you will need day before you want to start the experiment. It enables the atmosphere inside the tent to equilibrate and become completely anoxic. Next day, check the monitor what is the actual O<sub>2</sub> concentration (in ppm).

#### Preventive maintenance - once a week

- rejuvenate the catalyst (bake it in an oven at 125 - 200°C for 2 hours)
  - rejuvenate the desiccant (bake it in an oven at 125 - 200°C for 2 hours)
  - refresh hydrogen levels (let 1/4 of atmosphere volume off from a tent and then refill it with 95%N<sub>2</sub>/5%H<sub>2</sub> gas mixture)
  - clean the airlock seals (use wet sponge possibly soaked with soap or mild detergent, let it dried)
  - clean the chamber and disinfect if necessary (wash the desk with 1,5% chlorine bleach solution, wipe)
  - check the moisture trap on the vacuum pump and drain any visible water collected there
  - check the gas level/pressure in the bottles (approx. 40 psi (276 kPa))
  - check the tube coming out from the large dehumidifier for moisture/water and pour it away, but leave some water inside the tube to not allow air comes into the device

#### Preventive maintenance - once a month

- check the chamber for leaks
  - check both gas lines for leaks
  - grease the airlock door push pins
  - check the airlock seal to make sure the lip of the seal collapses all around the door opening

#### Preventive maintenance - once a year

- replace catalyst in HSR column and in Stack-Paks
  - calibrate sensors of CAM 12 (send them to Accela company)