Part I
Assembly of a bioreactor for *E.coli* fermentation

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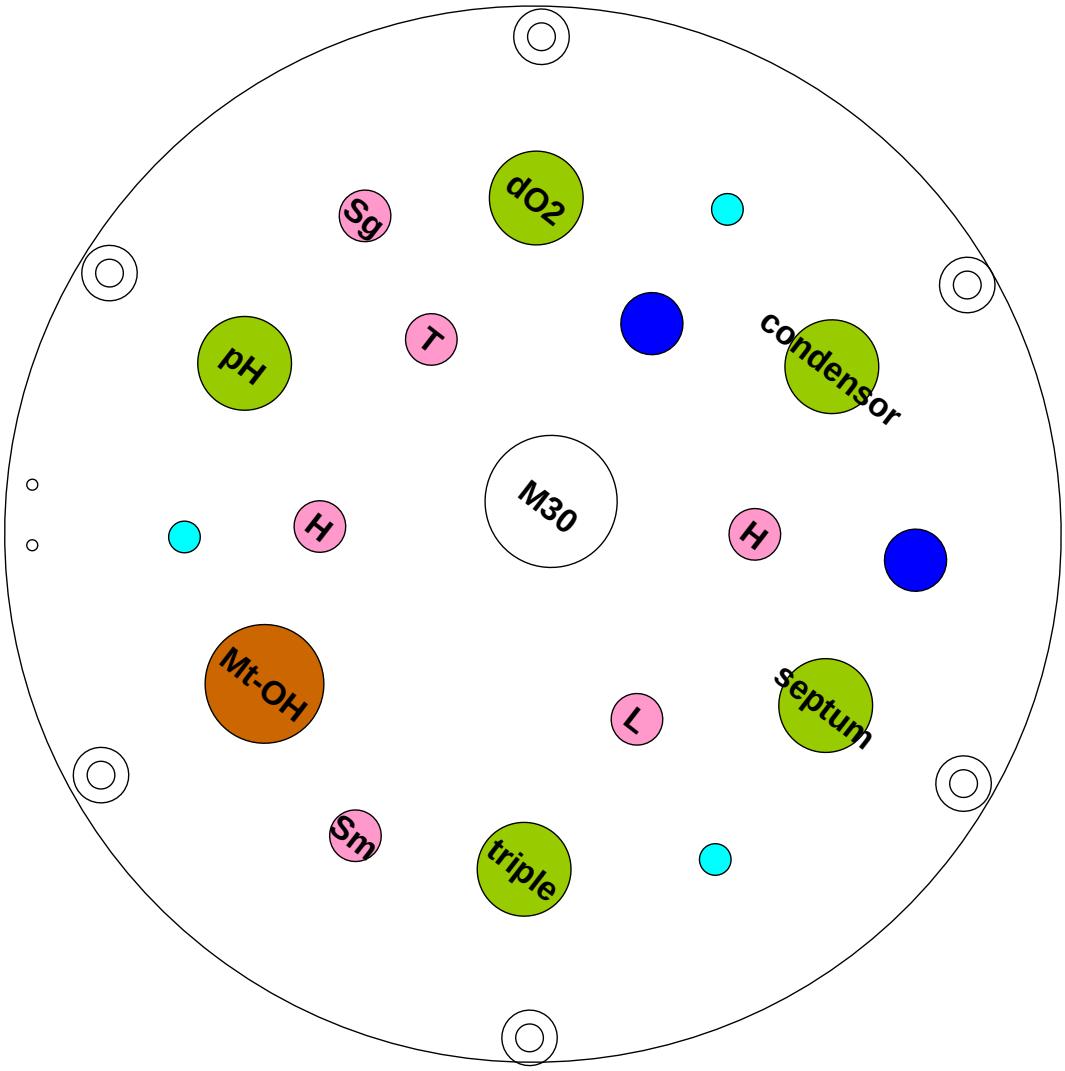
The 3 liter reactor for E.coli or Pichia fermentation:



¾'' port: Mt-OH sensor or blind plug

18 mm port: pH/dO2 sensor, condensor, septum, and triple

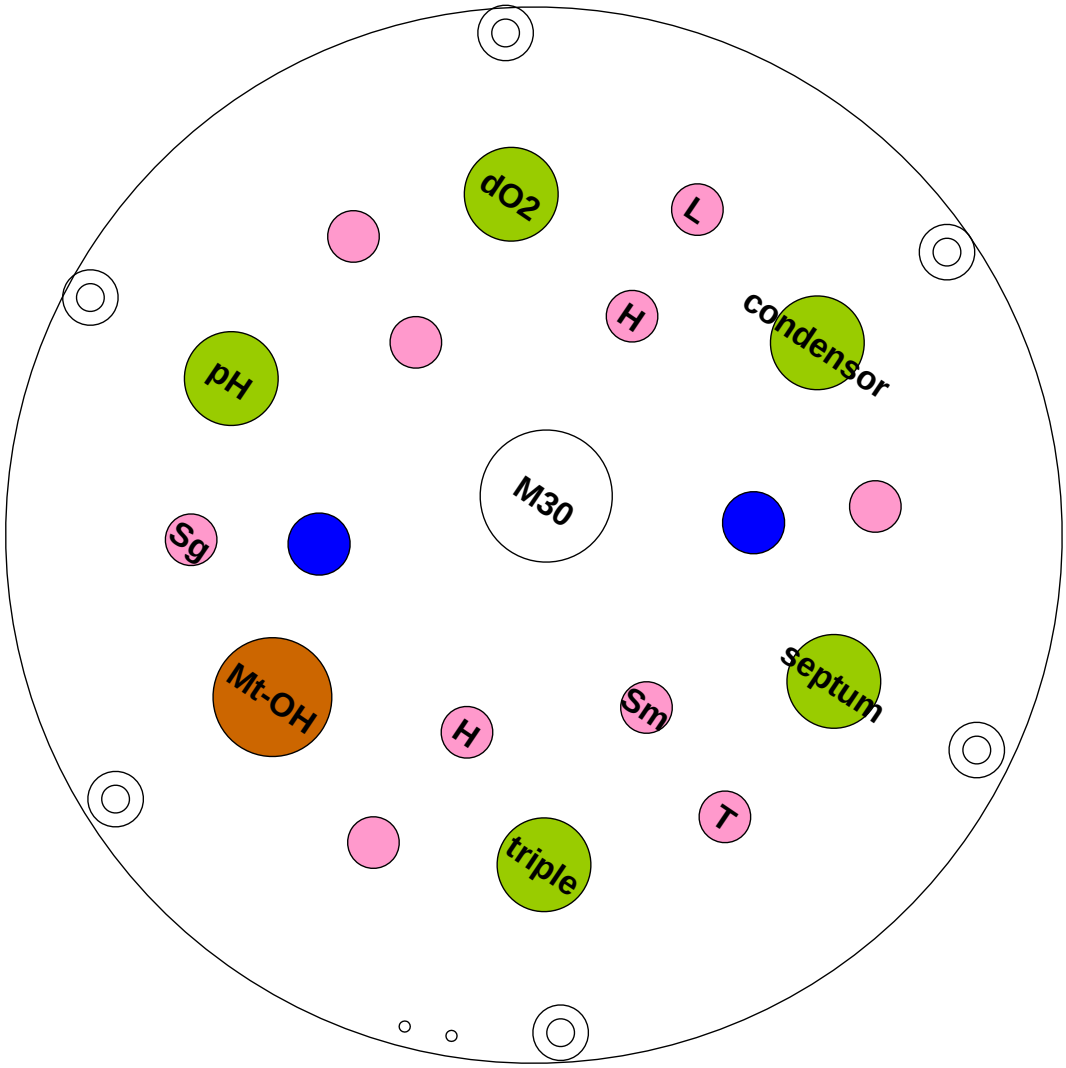
12 mm port: additional ports

10 mm port : Ring heat exchanger (H), temperature pocket (T), Sparger (Sg), or sample port (Sm), or antifoam sensor=label (L)

6 mm port: baffle (x3)

2 mm contact hole for level control

The 7 liter reactor for *E.coli* or *Pichia* fermentation:



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12 mm port: additional ports

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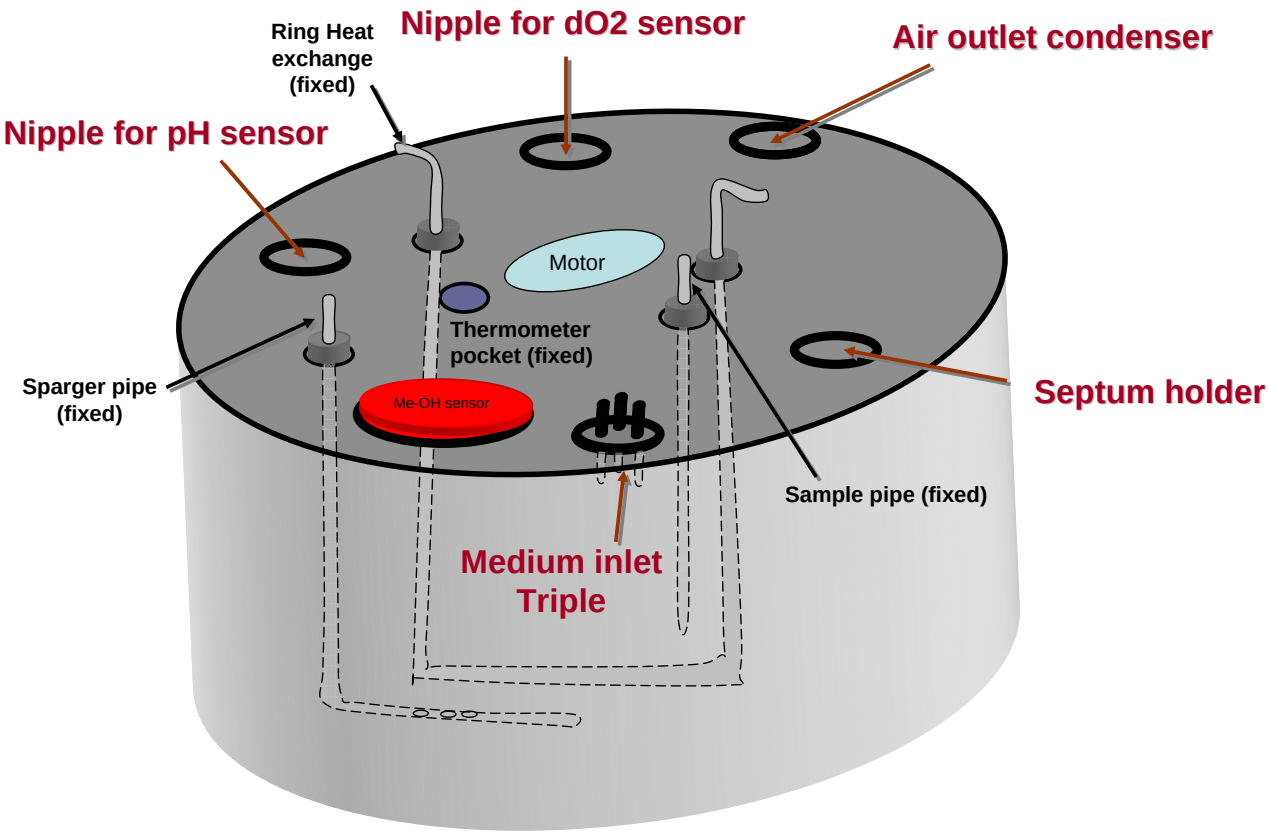
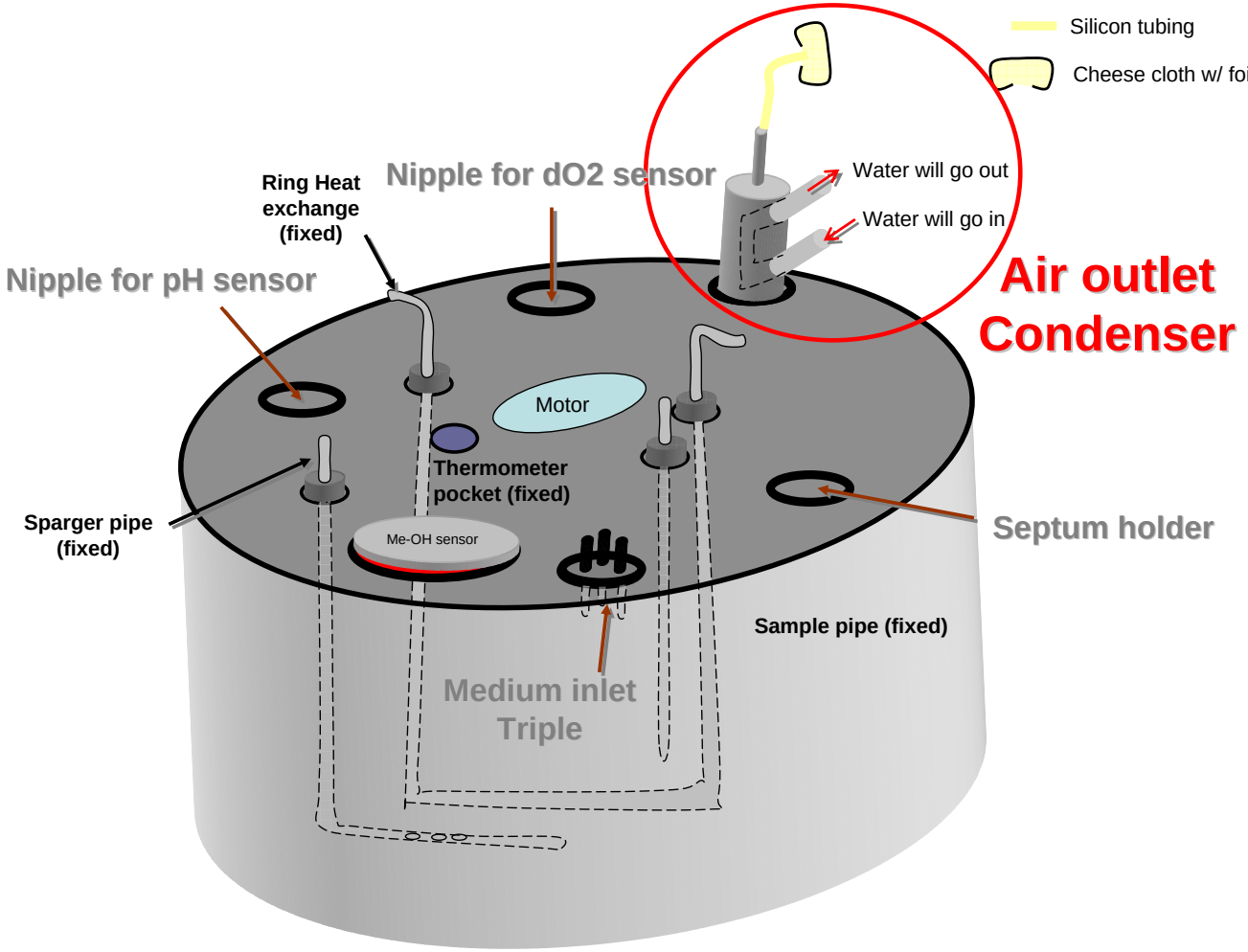
◦ 2 mm contact hole for level control



Step 1. Set up the head plate for *E.coli* fermentation

Air outlet condenser (Condensor)
Medium inlet triple (Triple)
Sparger pipe: installed already (Sparger)
Sample pipe: installed already (Sample)
Septum holder (Septum)
Nipple for pH sensor (pH)
Nipple for dO2 sensor (dO2)
Ring heat exchange water line: already installed
Motor
Thermometer pocket: installed already
Me-OH sensor: will not be used for *E.coli* fermentation. It is blocked by either a manually prepared rubber septum or a blind plug for 18 mm port (Z81301BD02).



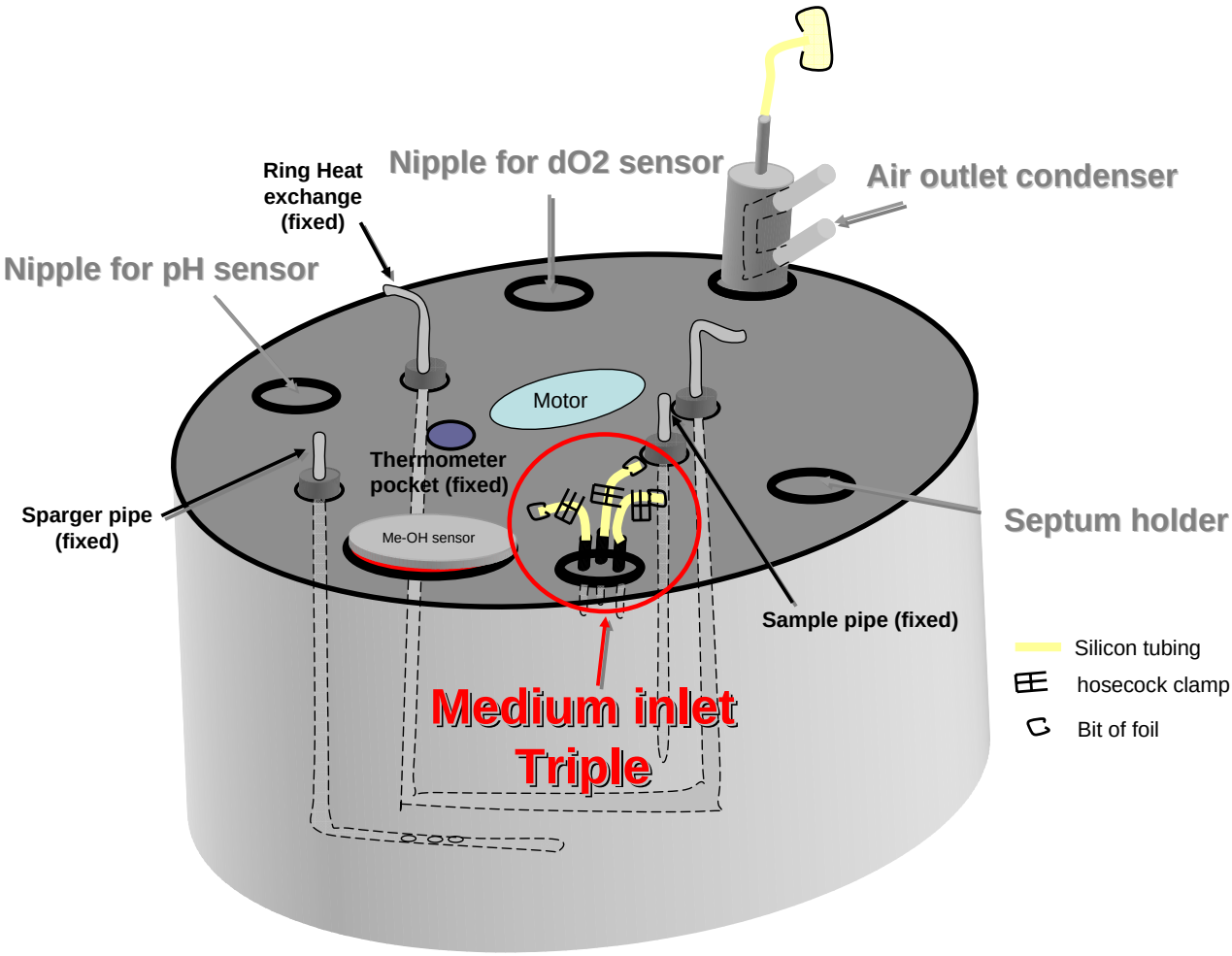
Step 2. Install the condenser

Condenser is the place where air is coming out. The air must be 1) cooled down to minimize liquid loss and 2) filtered before it is exposed to the environment.
The air is cooled down by the cooling water inside of the condenser. Filtering of the air needs installation of a filter at the end of the condenser.

Note: If the condenser is not assembled, assemble them first and install in the the head plate.

Preparation of the Condenser needs

1) Connection of the **silicon tubing (10-15 cm, already attached to the filter)** at the top of condenser.
2) Connection of **2-μm air filter (already attached to the tubing)** at the end of the silicon tubing.
3) Covering up the filter with **cheese cloth and foil.**

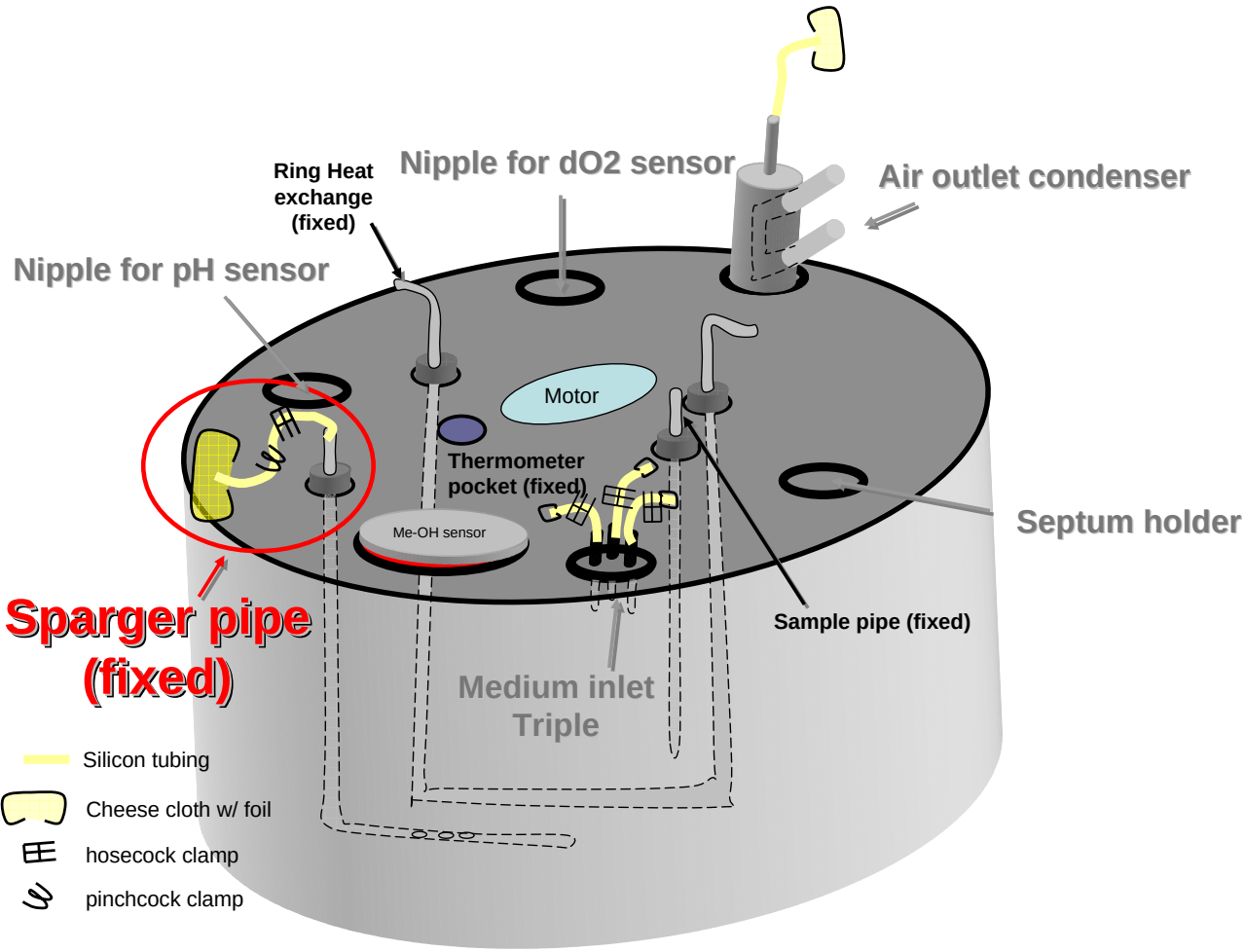


Step 3. Install the medium inlet Triple

Triple is the place where acid and base are added to the bioreactor during fermentation if necessary.

Preparation of the triple needs

1) Connection of the **silicon tubing (short tubing, 5-10 cm)** at the top of each triple.
2) Clamping of each tubing with one **hosecock clamp**. Totally 3 hosecock clamps will be used.
3) Cover the end of tubing with a **bit of foil.**

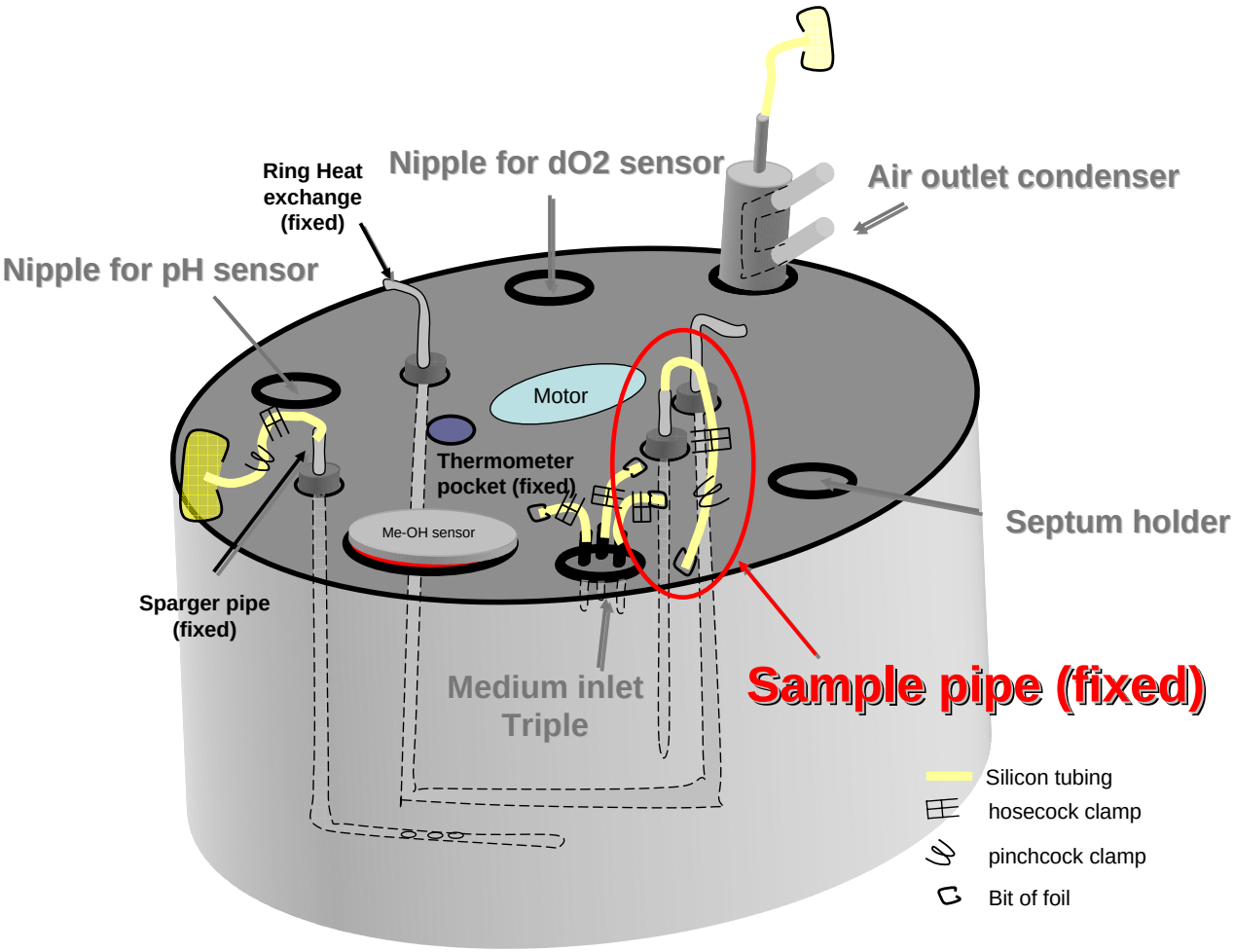


Step 4. Preparation for the Sparger Pipe

Sparger is the place where the air, oxygen, or nitrogen (if necessary) are coming into the bioreactor. The air needs to be filtered before it goes into the bioreactor. The end of the sparger pipe reaches the bottom of the bioreactor and release air bubbles through pores at the end.

Preparation of the sparger needs

1) Connection of the **silicon tubing (10-15 cm, already attached to the filter)** at the top of the sparger pipe.
2) Connection of **2-μm air filter (already connected to the tubing)** at the end of the silicon tubing.
3) Double clamping the tubing first with **a hosecock clamp** and second with **a day pinchcock clamp**. (same for all tubes which flow below the level of the media)
4) Covering up the filter with **cheese cloth and foil**.

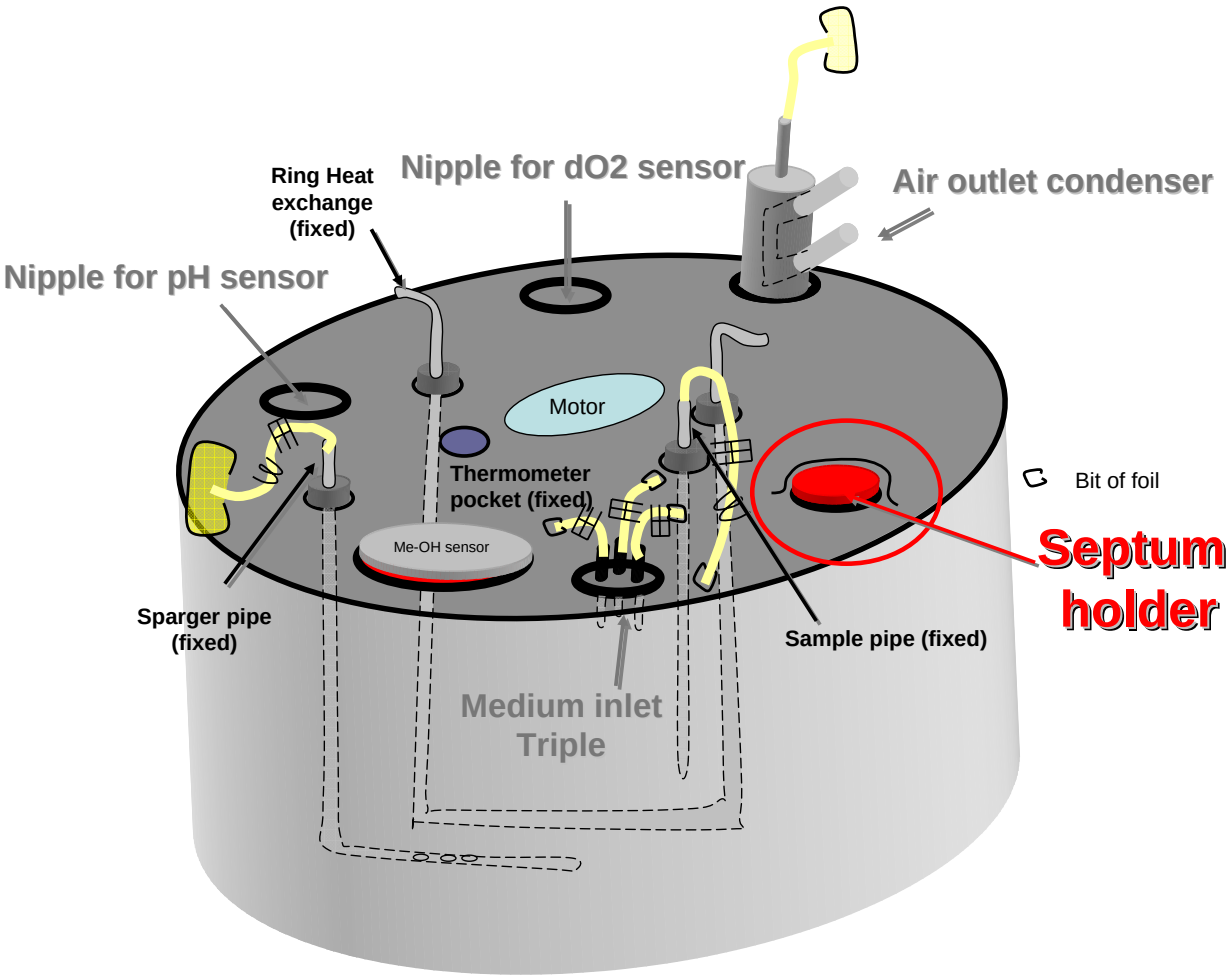


Step 5. Prepare the Sample Pipe

Sample pipe is the place for sampling during fermentation. Small volume (5-10ml) of culture can be taken aseptically by using a 10-ml syringe.

Preparation of the sample pipe needs

1) Connection of the **silicon tubing (10-15 cm, already connected)** at the top of the sample pipe.
2) Double clamping of the tubing first with **a hosecock clamp** and second with **a day pinchcock clamp**. (same for all tubes which flow below the level of the media)
3) Covering up the end of tubing with a **bit of foil**.

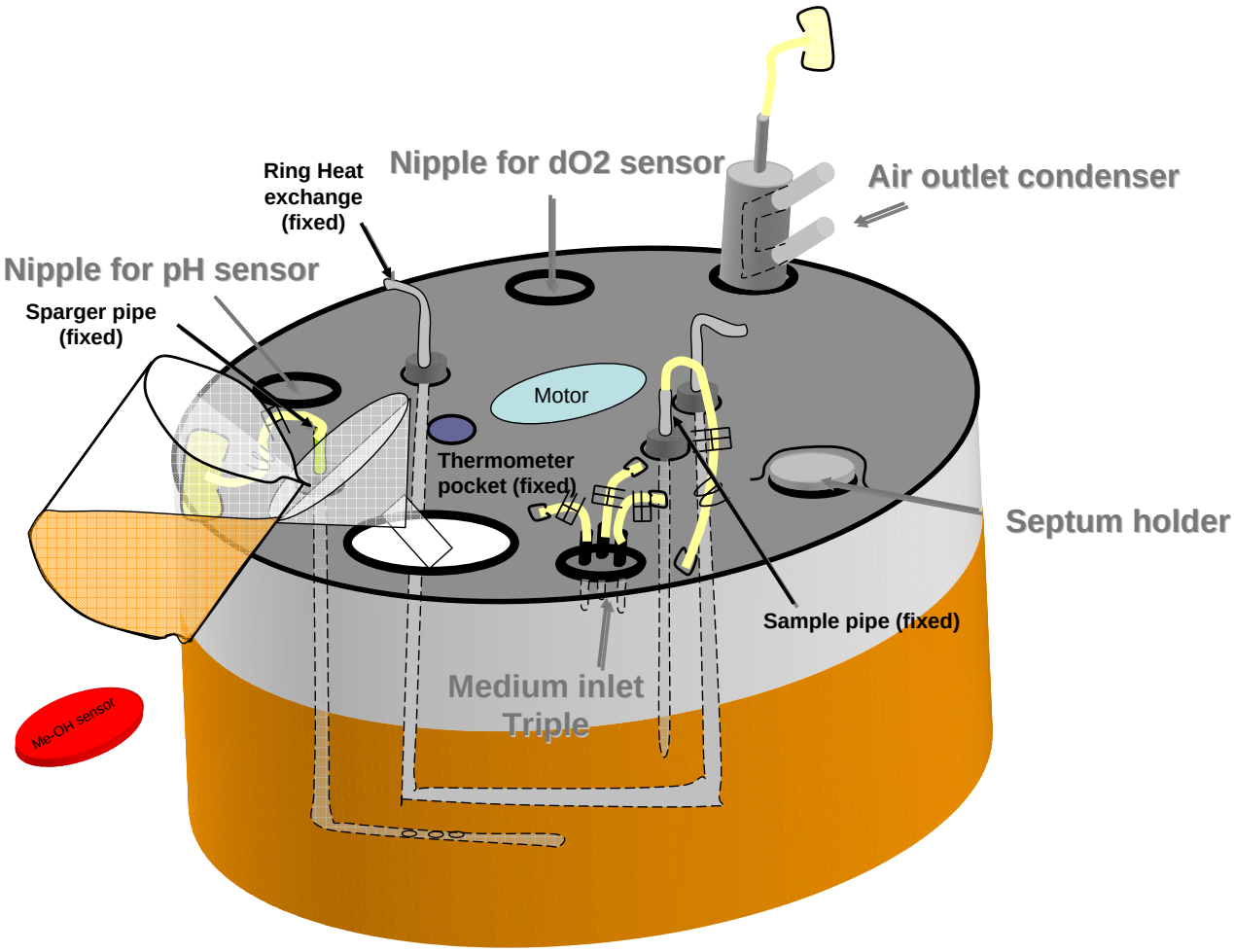


Step 6. Install the Septum Holder

Septum is the place where antibiotics or cells are added aseptically. Inoculation or antibiotics addition is done by using syringes connected to needle (No. G19 or G21) through the septum rubber cap.

Preparation of the septum needs

1) Check the condition of the septum and replace it if it is damaged.
2) Covering the rubber septum with a **bit of foil**.



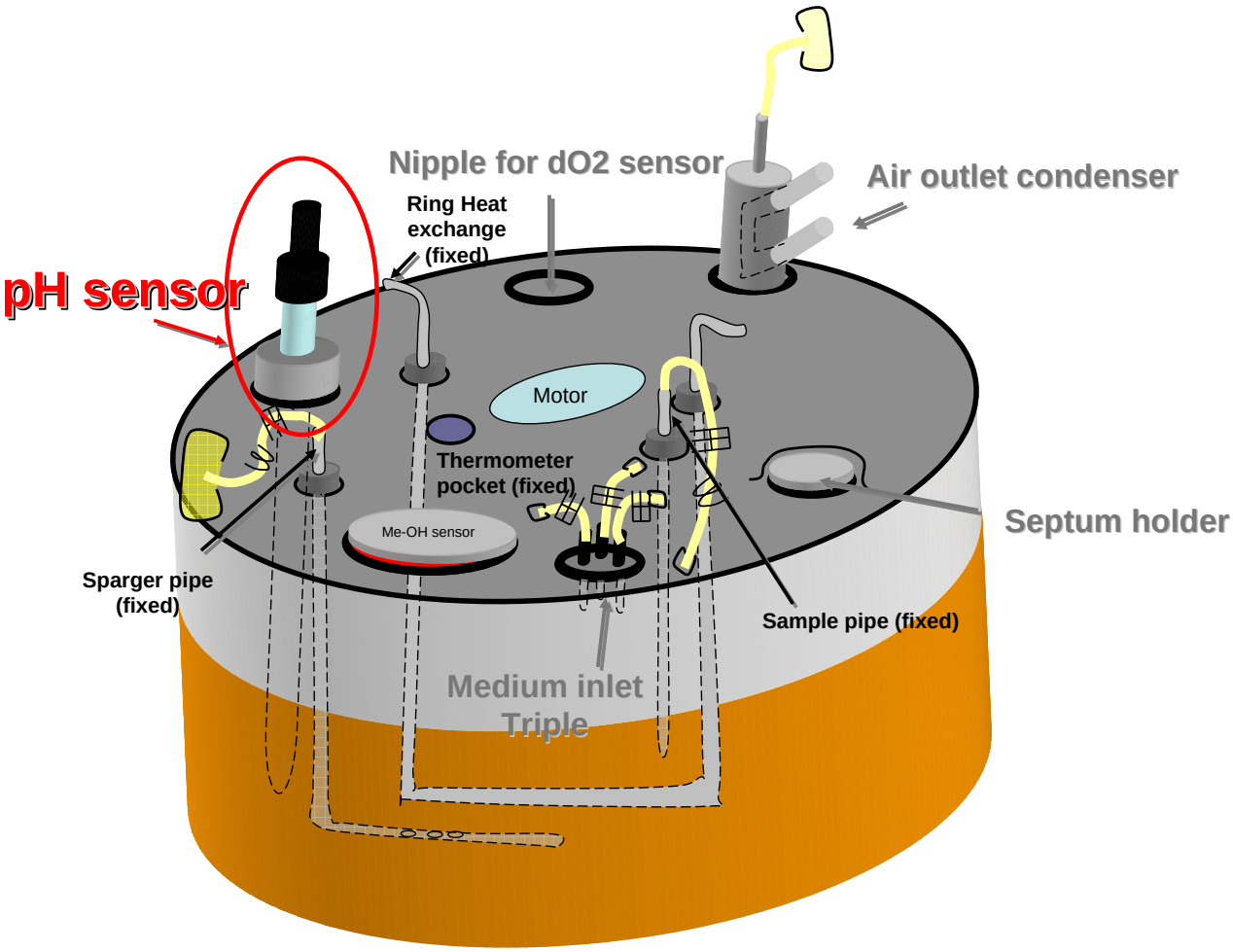
Step 7. Pour the medium in the the bioreactor

Prepare the medium

For 3 liter bioreactor: constant batch (< 2 liter of LB or TB)
For 7 liter bioreactor: constant batch (< 6 liter or LB or TB)

Pouring the medium into the bioreactor:

1) Open the Me-OH sensor cap and use **a funnel** to fill the medium into the bioreactor.
2) Close the port.



Step 8. Install and calibrate the pH sensor

pH sensor measures the pH of the medium during fermentation. It needs to be calibrated before sterilization.

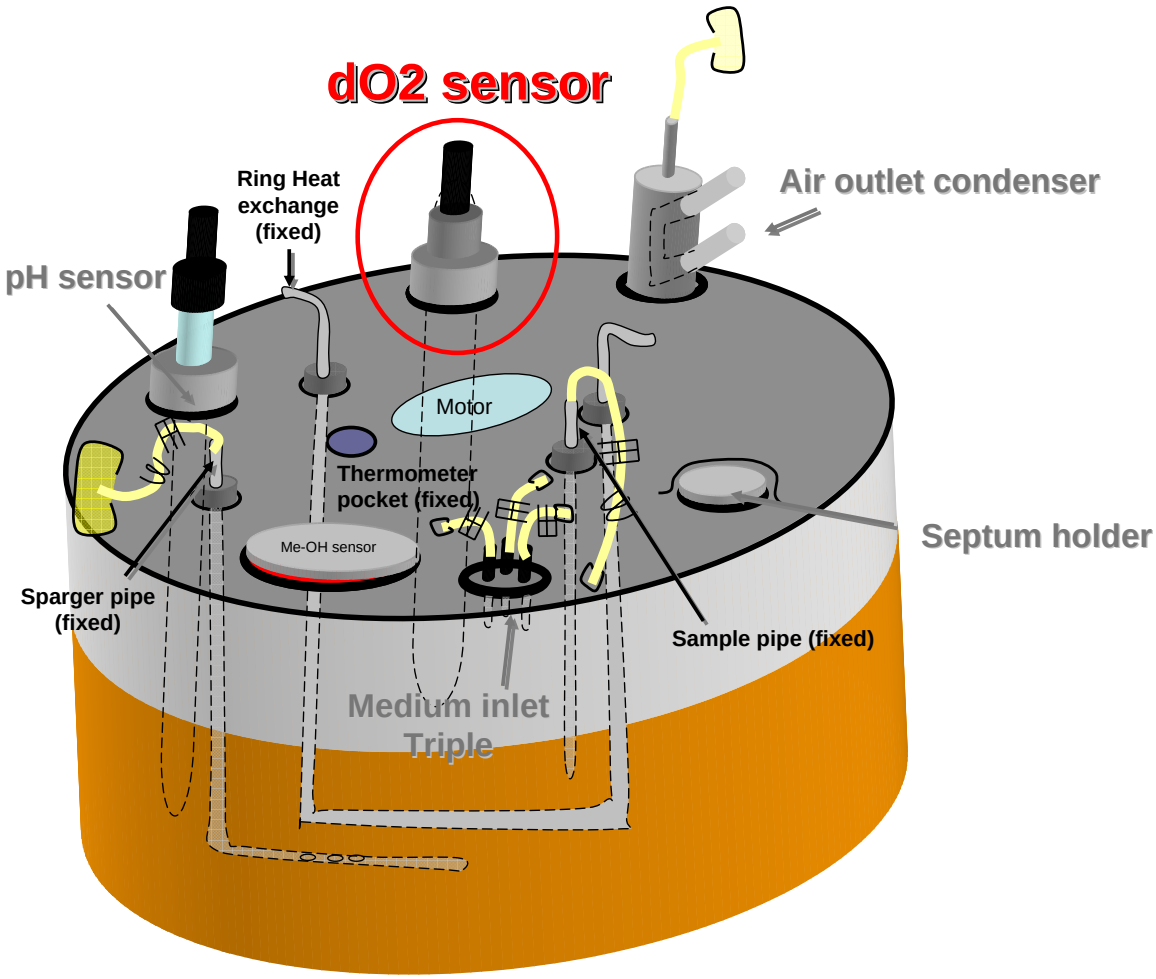
Installation of pH probe as followings.

1) Remove the pH sensor from the *storage buffer (saturated KCl,.filtered)*, wash thoroughly.
2) Connect the pH sensor into a pH cable connected to the ADI 1030 bio controller. Ensure the connection is tight.
3) Using pH standard solution pH 7.0 and pH 4.0, examine if the pH sensor is reading correctly. If not, calibrate the pH sensor.

pH calibration:

Main menu: Manual – Calibration – pH – Execute – Edit
Soak the pH sensor into the pH standard solution (7.0) and hit the enter button on the right.
(wait until it finishes [measuring])
Repeat with the 4.0 solution

4) Install the pH sensor into the pH port. Ensure the bottom of the sensor reaches the medium but not touching any parts.
5) **Cap** the top of the sensor.

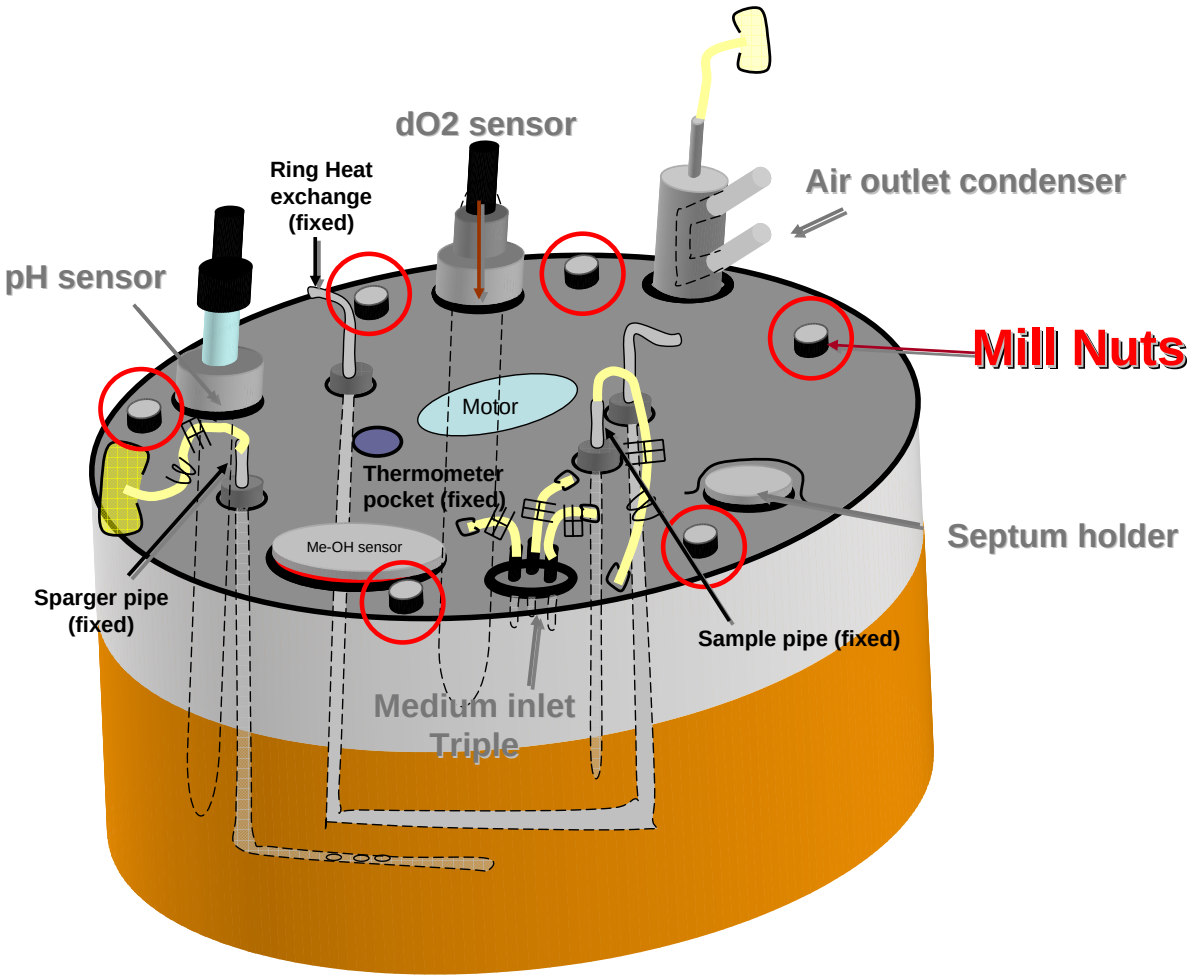


Step 9. Install the dO2 sensor

dO2 sensor measures the level of dissolved oxygen (dO2) of the medium during fermentation. Its calibration will be done after sterilization.

Installation of dO2 sensor as followings.

1) Remove the dO2 sensor from water, wash thoroughly.
2) Install the dO2 sensor into the dO2 port. Ensure the bottom of the sensor reaches the medium but not touching any parts.
3) **Cap** the tope of the sensor.



Step 10. Sterilize bioreactor

Instruction for sterilization.

1) Loosen **6 mill nuts** in the head plate.
2) Put the bioreactor into **an autoclavable tray**

- Sterilize the bioreactor **(45 min).**

After sterilization.

1) Take out the bioreactor from the autoclave.
2) Cool down the bioreactor.
3) Tight the mill nuts.
4) Bring to the fermenter room.