

WORKFLOW – Bioreactor for bacteria

1) Prepare the bioreactor for autoclaving – part 1

- a) Start with removing all connections:
 - i) Lift of the motor, put it on the side
 - ii) Unplug the two cables of the level sensor (red and brown/black connections)
 - iii) Disconnect the tubing from the water console. Click the ends of the tubes together to form closed loops
- b) Open the vessel
 - i) Undo the 6 mill nuts that hold the head plate. *Store the nuts in a box so you don't lose them.*
 - ii) Pull out the head plate by grabbing the stirrer and the side of the head plate. Carefully place it on the bench with the parts sticking out towards the air. *These are delicate parts that can bend if they are touching the bench.*
- c) Fill the vessel
 - i) Pour in about 25 ml of water to create some steam in the vessel during sterilization *If the vessel is autoclaved dry the pH electrode may be damaged.*
 - ii) You can also add your medium and sterilize it together with the vessel. *If you want to add medium after autoclavation it can be pumped in. Alternatively medium can be poured in through the septum port. Unscrew the port in a safety cabinet to keep it sterile.*
- d) Close the vessel
 - i) the head plate back and screw back the 6 mill nuts.
 - ii) Tighten the nuts cross-wise

2) Calibrate the pH sensor (page 19 chapter 4.2 in the manual).

- a) Connect pH sensor and temperature probe to the my-control and turn on the controller.
- b) Prepare two vials with pH calibration buffer (pH4 & 7 or pH7&10). *The vial needs to fit both, the pH sensor and the temperature probe*
- c) On the computer, start the WebUi by opening internet explorer and type in the ip address in the search field 192.168.1.242. Your screen will soon show the HMI of the my-control.
- d) Log in as a system engineer with password 0000.
- e) On the right side you will see 3 tabs, click on "sensors", then click on "pH"
- f) On the left side it will now pop up a field with information related to pH. Select the tab "calibrate" and click on "2 point calibration"
- g) Type in the temperature that you see in the right field of the screen. *Wait until the temperature reading has stabilized.* Press the check mark.
- h) When the pH has stabilized in the field of pH in the tab of sensor on the right side of the screen, type in the pH you're measuring.
- i) Repeat for the second pH point
- j) When you calibrated both pH you will have a look at the slope and offset. *These values can be used to keep track of the probes health.*

3) Prepare the bioreactor for autoclaving – part 2

- a) Insert the calibrated pH sensor
 - i) by screwing it into the head plate and
 - ii) adding the protective plastic cap onto the end that is sticking out of the vessel.
- b) Insert the DO sensor (see also *Lumisens* manual)
 - i) Remove the yellow cap from the glass tube.
 - ii) Separate the glass tube from the sensor head. *This is a magnetic coupling.*
 - iii) Screw the sensor into the head plate
 - iv) Screw the protective cap onto the end that is sticking out of the vessel

- c) Adjust the level sensor
 - i) Gently push the sensor down until you only see a small piece of the plastic covering
 - ii) Fasten the screw at the base of the sensor
 - d) Close all openings with tubing
 - i) Use the tubing that came with the system.
 - ii) Cut the tubing short, they can stay on the head plate and be re-used for a couple of runs.
You will use connectors to add longer pieces of tubing for your addition bottle/gases
 - iii) Add pieces of tubing to the inlets, starting with the triplet addition inlets. *If the tubing is bit loose, tighten with zip-ties (part of the start-up kit).*
 - iv) Clamp the tubes – use one clamp per tube. *Clamps can be found in your start-up kit and new can be bought very cheap on amazon. Do not clamp the tube of the condenser!*
 - v) For the sparger (the “hockey stick”), add tubing with a filter at the end. Place the clamp between the inlet and the filter.
 - vi) Make sure all the clamps are closed.
 - vii) Make sure there is no clamp on the tube of the condenser. It is used for pressure release
 - e) Protect open ends with Aluminum Foil
 - i) Make small “hats” of foil around the ends of the filters including the filter on the condenser.
 - ii) Cover the level sensor and the opening for the temperature sensor
- 4) Prepare the addition bottle for autoclaving**
- a) Add your alkali, acid or feed that you wish to autoclave
 - b) Put a ‘foil hat’ on the filter
 - c) If you want to use the pump, connect the thinner tubings to the bottle and the inlet
 - d) Place bottle in holder
- 5) Autoclaving**
- a) The autoclave is located in building 404 and operated by BredaBlick.
 - b) Autoclaving takes place on Tuesdays and Thursdays and the autoclaving goods must be delivered to the autoclave or placed in the before 9:30.
- 6) Starting a run**
- a) Connect all the sensors
 - i) Add a little water to the tube where the temperature probe goes. Insert the probe.
 - ii) Connect the cables of the level sensor. *Red goes to the level sensor, the brown/black to one of the two 2 mm holes on the head plate.*
 - iii) Remove the plastic caps of the DO and pH sensors and connect the cables. *The DO sensor needs to self-calibrate for 30min before readings are stabilized.*
 - b) Connect the heating blanket
 - c) Start Lucillus before you do your inoculation to be sure you record your data.
 - d) Start your process by adding your bacteria either by pumping it in or syringe through the septum.
 - e) Taking samples during the run can be done through a dedicated tubing with filter