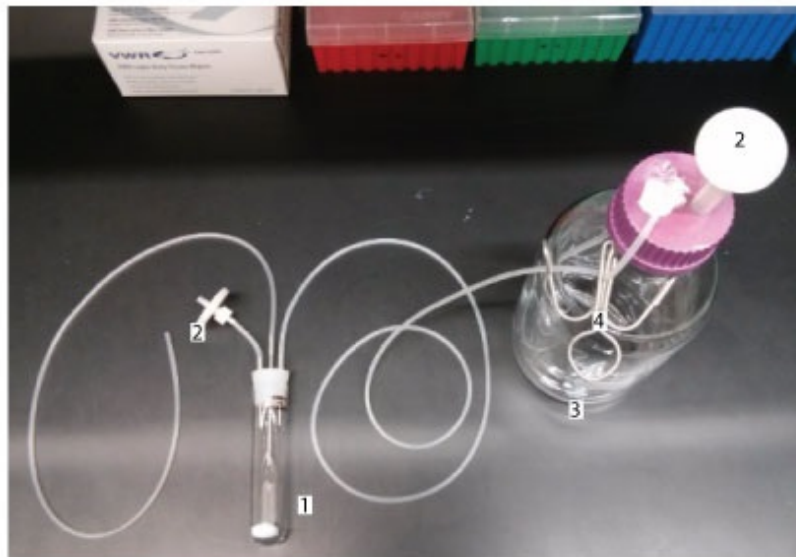
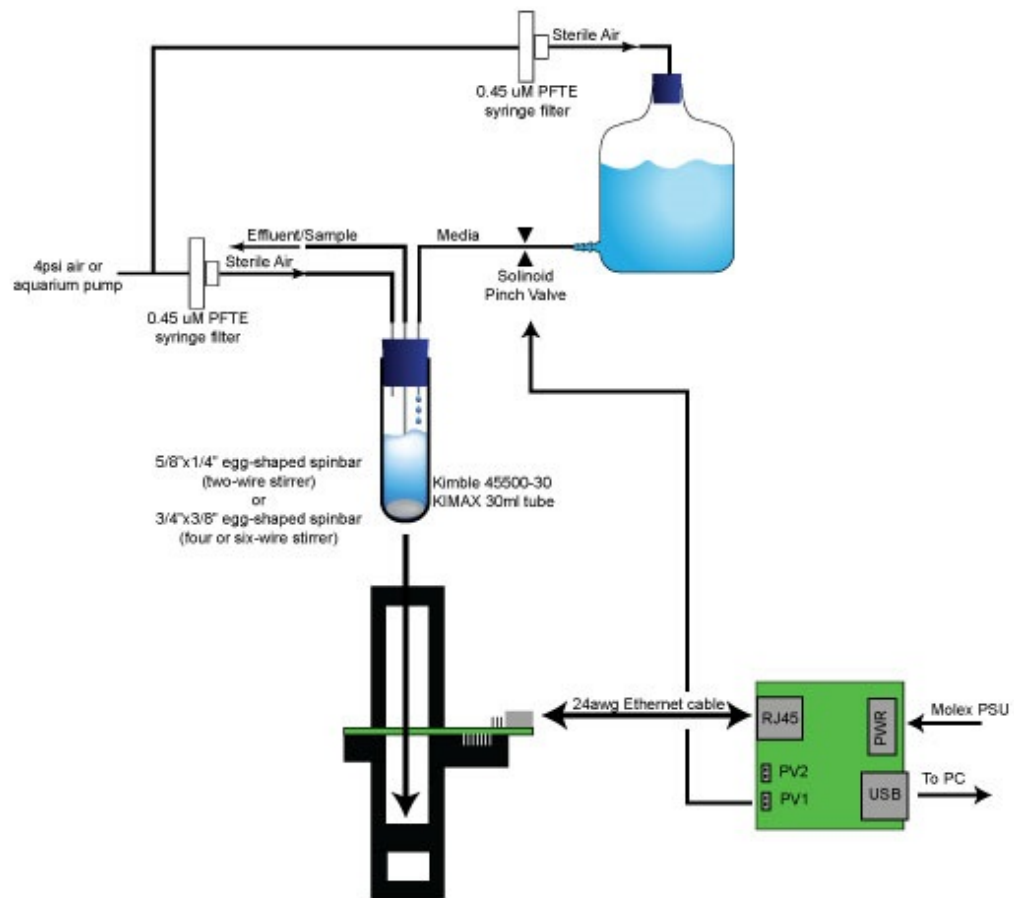


#### Day 0:

1. Make fresh bottle of media.
  - a. 500mL should easily be enough for 24 hours of syphonstat.
  - b. Ensure you use a bottle that has a pour ring as this is needed to ensure that enough pressure can be produced in the bottle when the lid is tightly screwed on.
2. Prepare overnight cultures.
3. Assemble closed system as shown in the hookup guide.
  - a. If your media is autoclavable, add it now and make sure to clamp off all tubes going into the media bottle (loosen the lid/stopper to allow gas to vent)
4. Autoclave the closed system (Be sure to foil over any open ends including the PTFE filters)

#### Day 1:

5. Add media using sterile technique if not autoclavable.
6. Insert culture tube into culture chamber and place media bottle 1-3 feet above the culture tube.
7. Insert media line into pinch valve and unclamp media and air lines
8. Attach air line to media bottle
9. Check for pinch valve leaks
  - a. If the media is passing through the valve check that the tube is in the normally closed (top) side and that you're tube is not undersized (very common). If you have undersized tube you can use a few layers of lab tape to make the valve smaller.
10. Press the override button on top of the pinch valve (or temporarily remove the tube from the valve) until the culture tube fills with media up to the effluent port.
11. Attach air line to culture chamber
12. On the PC remove any old log and dat files
13. Attach Power to the syphonstat controller board
14. If inoculating dense or a high volume of cells
  - a. Start the software and wait at least 1 minute for the system to blank
  - b. Inoculate by piercing the stopper with a syringe loaded with inoculum
15. If inoculating with few cells ( $OD \sim 0$  when diluted to 15ml)
  - a. Inoculate by piercing the stopper with a syringe loaded with inoculum
  - b. Start the software and wait at least 1 minute for the system to blank



1. Tube and stir bar
2. PTFE filter
3. Media bottle
4. Tube clamp