Turbidostat Protocol

2 Days Before

1) Reserve desired turbidostat on outlook calendar
2) Ensure that you have a recent calibration file (within the last month)
    a. If not, prepare an overnight culture of any cells and see calibration protocol

Day Before

1) Check that incubator is on and is at correct temperature.
2) Autoclave and otherwise prepare turbidostat supplies as needed: bottle-top squids, vials, sterile water (for washing), media, antibiotics, any other media additives
3) Perform calibration if needed (see calibration protocol).

Setting up and starting the run

1) Turn on the DC voltage source for the IR emitter/detector pairs (table top, orange button). Confirm that the voltage is set to 6V for both channels. Do this at least 1h before blanking the instrument or taking any measurements.
2) Check waste carboy (on cart between turbidostats), if necessary change out and put ~25ml bleach in the bottom of fresh carboy, and empty full carboy (rinse and put in dish cart). Double check that all waste outlets are positioned in the carboy lid, and that the end of the tubing is suspended above (not in) the waste.
3) Aliquot media (with necessary antibiotics/additives) into the turbidostat vials you plan to use (17 mls per vial). For each vial:
    a. Make sure there is a stir bar
    b. Wipe the outside surface of the vial with a kimwipe to remove any fingerprints/smudges
    c. Label the vials
4) Place the vials in the turbidostat while you finish setting up… preferably you will leave them in for an hour or more to allow the media to prewarm to 30C.
5) Modify the file "Initialize.m" for your experiment. The variables you will mostly likely want to change are:
    a. `io.num_tubes` (the number of tubes/turbidostat holders you are using)
    b. `io.expt_length`
    c. `io.targetOD` (specifies the clamped OD for all holders – leave any empty holders set to 100)
6) Clear the workspace and run Initialize.m to set up the experiment:
    a. `clear; close all; delete(daqfind); delete(timerfind);`
    b. `io = Initialize('calibration file', 'blanks file')`
7) If successful, this should create a structure "`io`" in the current workspace that contains all of the daq, analog output, and analog input device objects necessary for controlling the instrument.
8) Prepare your media for the experiment – sterile-filter into a 1L bottle. Attach an autoclaved bottle-top squid. To do this, use tweezers to hold onto the black cable tie on the tubing and guide the tubing into the (flamed) bottle mouth.
9) Attach a sterile 0.2um filter to one of the sealed media inputs (for vent).

10) Prior to connecting the media bottle to the turbidostat, you will need to run sterile water through the media input lines for approximately 5 minutes. The prior user should have cleaned the lines and left them in 70% EtOH.
    a. Attach the media input lines you will be using to a bottle of sterile water with squid. Flush the intake lines with sterile water for five minutes.
    b. To control the intake pumps and execute the wash steps, you will need the following commands:
    c. `pumps = [17 15 13 11]`
       (for the first four "A" pumps, adjust this list of pump lines according to what you need, using the chart on the side of the incubator)
    d. `for k=1:numel(pumps);`
       `putvalue(io.relaybox2.Line(pumps(k)), 1); end`
       This switches pumps on. If it works, you should here clicking as the pumps switch on, and media should move into the tubing…
    e. `for k=1:numel(pumps);`
       `putvalue(io.relaybox2.Line(pumps(k)), 0); end`
       This switches pumps off.
    b. Flush the outtake lines with sterile water.
        i. Change vials to fresh sterile vials (no stir bar necessary)
        ii. Fill the vial with sterile water with the commands to turn on the intake pumps
        iii. Once the level of sterile water has exceeded the level of the outtake line, turn on the outtake pumps with the following commands:
            1. `putvalue(io.dio_daq(2),1); %switch off`
            2. `putvalue(io.dio_daq(2),0); %switch on`
        iv. Flush the outtake line with sterile water for 5 minutes
        v. Turn off the intake pumps and the outtake pumps
11) After the lines have been flushed with sterile water, make sure all pumps are off. Then attach the desired media input lines from the turbidostat to the input lines on the media bottle. Use good sterile technique – I usually wipe my gloves with a little bit of 70% EtOH.
12) Flush media through the lines (again using the above pump commands)– usually for ~5min.
13) Run the outtake pump for 5 mins to test pumps and also take volume out of vials:
    a. `putvalue(io.dio_daq(2),1); %switch off`
    b. `putvalue(io.dio_daq(2),0); %switch on`
14) Mark the level of media on the outside of the media bottle(s) with a sharpie (useful for estimating the rate of media use later), and switch all pumps off.
15) Now the preliminary set up is done… I usually estimate that steps 1-14 take 1.5-2h total. At this point you are ready to start the run, but you can also leave everything for a few hours while you prepare your cultures.
16) Getting ready to start the run… inoculate the desired amount of culture in each turbidostat vial. Use good sterile technique. If applicable, measure OD of inoculated cultures (aiming for just below your OD setting on turbidostat run)

17) Move vials to turbidostat and move green caps to the culture tubes and place in turbidostat holders. Make sure that the tubing that is attached is numbered to match the turbidostat holder that your vial is in.
    a. If you need lights on during the run use the following command:
       `putsample(io.ao[1 1 1 1]);` (for as many vials as needed)
    b. To turn lights off, use the following command:
       `putsample(io.ao[0 0 0 0]);` (for as many vials as needed)
18) To start the run, issue the following commands in the matlab window:
    a. `startT = now();`
    b. `Run(io, startT);`
19) The run should begin! If nothing plots, try closing the plotting window once – it should reappear with the OD values graphed.
20) To pause (i.e. collect samples) and restart, you can use the following commands:
    a. `stop(timerfind)` %turns all pumping off
    b. `start(timerfind)` %re-starts pumps and graphing.
21) Note that the run will NOT end automatically. You will need to repeatedly check on the run to make sure you are not depleting media/running the lines dry. *There are two important consequences for running the lines dry: 1) they become more difficult to prime/fill with media on the next run, and 2) if a vial is over OD, the pumps will continually switch on to try and add media. The pumps are not designed to be run continually, will overheat, and burn out if you do this. Not only does it damage the instrument, but presents a fire hazard risk.*

Ending the run and cleaning up….

(1) To end the run, issue the following commands:
    a. `stop(timerfind)`
    b. `stop(io.ai)`
(2) All of your data should be contained in the most recent (time-stamped) workspace saved out to disk.
(3) Turn off the stir plate
(4) Turn off the DC voltage source
(5) Switch in fresh tubes to the turbidostat holders; use good sterile techinique to swap vial tops onto fresh tubes. Unhook your media bottles and connect the media input lines to the squid on a bottle of sterile water.
(6) To clean the turbidostat tubing… flush with the following: ddH2O, 10% bleach, ddH2O, 70% EtOH, swapping fresh tubes in after the bleach and also before the final 70% EtOH wash. Note that if you forget to put water in between the bleach step and the EtOH step, a bunch of gross crystalline white stuff will precipitate out in the lines. You can flush the media input lines using the commands given in step 10 (above). To turn the outtake pump on/off (to flush the outtake lines), you'll need the following commands:
    a. `putvalue(io.dio_daq(2),1); %switch off`
    b. `putvalue(io.dio_daq(2),0); %switch on`
(7) To clean the vials and stir bars: Scrub thoroughly with deionized water, squirt 70%EtOH and ddH2O water through the tubing on the vial top. Invert in rack, and let dry thoroughly

(8) To clean the bottle top squids – flush with ddH2O, then 70% EtOH, and dry using line air. Once the squids and vials are dry, they can be autoclaved on gravity cycle.
(9) Make sure all pumps are off, and double-check that stir plate and power supply are turned off.