

Botstein Lab Chemostat Manual

Before you start:

- Choose your strain
- Decide what limitation you are going to use
- Decide what dilution rate you are going to use or if you are going to do a growth rate series
- Decide what exactly you want to do...i.e. evolution, steady state sampling, pulses etc

SignUp

Sign up for chemostats on the board outside of the chemostat room. Be sure to include how long you will be using the spot for. Do not go over your time as other people plan to use the chemostat based on your signup. Read all notes before starting to make sure the bank you are signing up for is available.

Carboy Preparation

Autoclave an empty carboy (use the liquid cycle to prevent breakage). Foil all ends of tubing as well as the blue port. Mix up all the media in a separate carboy and stir well to dissolve everything (you can use salts, vitamins, and metals from stock solutions, but you should use granulated glucose). Attach a bottle top filter with the cotton filter removed (see suppliers at end) to a 100 ml bottle. Do this as sterile as possible. To take out the little white filter, use forceps that have been sitting in ethanol. Attach to the filtration port in the carboy, hook the vacuum up to the big top filter, and filter it all in. This will take ~15 minutes. Although the filters are nominally for only 1 L, we have not had any contamination problems associated with filter failure. (although this can happen so save your 100mL bottle for a few days to ensure nothing grows in it). Be sure to tell Sandy if metals or vitamins are running low.

Also, decide how you will arrange the media flow to the chemostats. If you want to run multiple chemostats off one media carboy, you need to make a Y connector out of scrap tubing. Make sure you put the right connectors on all the

ends. If you want to run chemostats off multiple media carboys, you will need a more complicated branching connector. Make the connector piece and wrap foil over all the ends. Don't hook it to the chemostats yet since it will interfere with setting them all up.

The Chemostat Vessel

For each chemostat, you have a culture vessel, O-ring, stirrer/probe/aeration/port assembly, clamp, effluent drop tube, various probes, pump tubing, air inflow, air release, and effluent tubing.

Note: pump tubing size matters. The calibrations for dilution rate (below p. 23) are for Masterflex 95809-42 only.

Assembling the vessel

Even if it looks like the last person to use the vessel cleaned it, rinse it again with DI water before you use it. Never use soap on any chemostat item. Rinse all lines thoroughly with DI water and replace any tubing that looks old and/or yucky. Keep the little plug over the temperature probe when washing and autoclaving. If using pH probes, keep the cap on when washing and autoclaving.

Make sure the stirrer is snug in the housing. If it is not, snap it into place. Push the little o-ring against the joint to hold the stirrer in place. Place a big o-ring in the rim of the glass vessel. Put the stirrer assembly into the vessel. Open up the clamp, fit it around the neck of the chemostat, and clamp down until it no longer moves easily. Make sure the clamp is seated properly on the rim of the vessel. Set the assembled chemostat in the carrying rack. Ensure that the assembly isn't pressing against the bottom of the vessel. It should be flush with the bottom.

Determine which probes you want to include. We currently have pH and dissolved oxygen. Sign up for probes on the probe log. Always use the next numbered probe (i.e. if the last person used #2 use #3). Unscrew the appropriate size plugs and screw the probes into the ports on the top of the vessel. Remove the plastic caps from the DO probe. Set

the plugs and caps aside in the appropriate bucket so we can find them later. Keep the pH cap on while washing and autoclaving. The pH and DO probes must always be kept in water, buffer, or media. Do NOT store the probes for an extended period of time in water. You can only store them in water when autoclaving the vessel and while the DO probe re polarizes overnight..do NOT leave them in water for multiple days!! When not in use, they should be capped and placed in the rack with the others.

Determine whether or not you will be controlling pH during the run. If so, you will need two "media port" inputs with pump tubing.

Use the marked clear plastic ruler to calibrate the working volume. There are separate rulers for the commonly used settings. Make sure you use the correct ruler for the type of vessel you are using, ACE or HT. We typically run the chemostats at 300 ml. The markings on the ruler are for the height of the top edge of the effluent tube relative to the top of the casing it sits in. The height depends on the number of rotors and the number and type of probes. To adjust the height of the tube, unscrew the small screw on the side of the casing. Adjust the tube to the appropriate height and tighten the screw back down.

If you are using the oxygen probe and/or pH, don't adjust the effluent tube height to the working volume. Instead, push the tube almost all the way to the bottom of the vessel. This maintains sterility when you go to eject the water before you start the chemostat. If you do this, make sure to clamp the effluent tube when autoclaving so water doesn't flow out while autoclaving.

Attach a piece of medium bore tubing to the effluent tube. This should be long enough to reach the effluent vessel in front of the chemostats.

Attach a piece of medium bore tubing to the air input port. Put a non-locking luer fitting on the end.

Any free ports need to be plugged. You can make plugs out of scrap ~1 in small or medium bore tubing with silicone glue injected in one end. A less desirable option is short pieces of tubing with clips. If using clips, make sure they are not broken before autoclaving. Only use clips if you need that port for something, otherwise use plugs!

Keep tubing clips open during autoclaving to allow steam venting. If the vessel is full of water, clip the effluent tube and the air tube to prevent ejecting the water.

Cover all the tubing ends with aluminum foil. Put a piece of autoclave tape over some of the foil if desired.

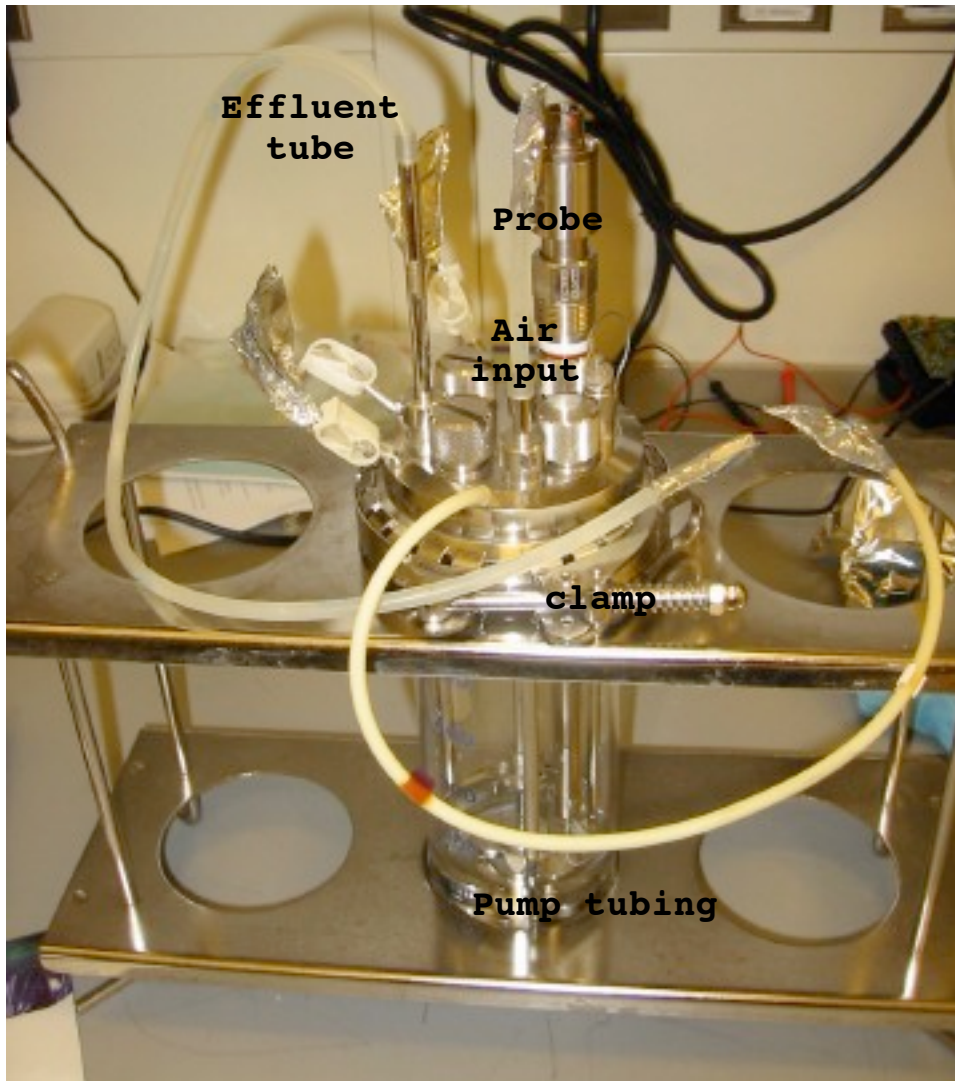


Figure 1. Assembled chemostat vessel.

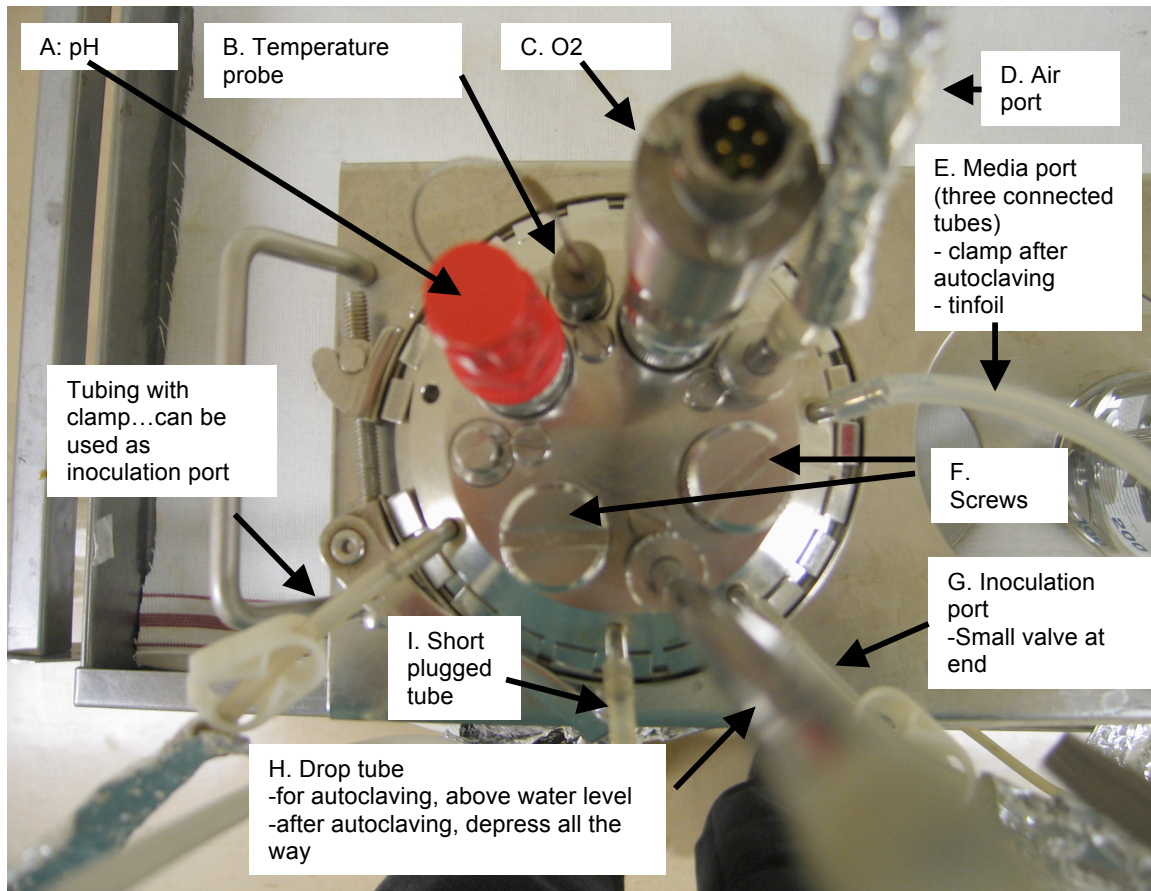


Figure 2: Top of assembled vessel

Calibrating the pH probe

pH probes have to be calibrated before autoclaving.

To calibrate the pH probe hook up the probe to the bank that you are signed up for. To hook up the probe unscrew the cap and screw the probe into the connector. Make sure to write down the number on the probe that corresponds to the position on the bank. The vessel with that probe has to go in that spot for your run.

Go to the calibration page by:

- Hit 1 - Fermenter menu
- Select the individual fermenter
- Hit 2 - Parameter menu
- Hit 3 - pH
- Hit 2- calibrate

The screen will show a high and a low reference. Scroll to the high reading for the probe (i.e. not where it says 7, but rather the mV reading). Place the probe in buffer 7 solution. Wait for the reading to stabilize (about 30 sec to 1 minute). Hit enter. Remove the probe and rinse with dH2O. Place probe in buffer 4 for the low reference read. Wait for the read to stabilize and hit enter. The readings should be close to the actual pH of the buffer...if they are not, there is a problem with either the probe or the connection. Check the probe by using another connection. Check the connection by using another probe. If the probe is bad, give it to Sandy. If the connection is bad, place a bit of tape on the connection and tell Sandy. Cap the probe after calibrating.

Autoclaving the chemostats

If you are running chemostats with oxygen probes, fill the chemostats with water autoclave them for 15 min on the liquid cycle. Make sure to add enough liquid that the probes do not dry out. Also, put the autoclave probe in a beaker of water.

If you are autoclaving the chemostats dry, use the bedding setting for 15 min.

Close all the tubing clips once the chemostats come out of the autoclave.

Extra tubing should have both ends wrapped in foil before being autoclaved for 15 minutes on the bedding cycle.

Setting up a run

Assemble the sterile chemostats and media vessels for your run. You want to start setting up the day before you inoculate, especially if you are using the dissolved oxygen probe. You need to start the air supply, connect the media, connect the probes, fill the vessel with media, and program it. Then you can inoculate.

Polarizing the dissolved oxygen probes

The oxygen probes must polarize for at least 6 hours before you begin. Put each chemostat into a holder on the machine. Line up the red line on the connector with the red line on the DO probe. Plug the dissolved oxygen probe into the gray cord by pushing down and twisting the connector. It should lock into place. Make sure the main power to the machine is on. Leave plugged in for 6 hours, best if left overnight.

Setting up the vessel

Plug the red temperature cord into the plug on the chemostat vessel (see Figure 2). The red dots should line up. On the newer model, the metal probe on the red cord should be threaded into the metal casing on the vessel (Important! Unlike on the old machine, which assumed a high temperature if you forgot to plug in the probe, with the new probe, if you forget to attach it, the machine will read room temperature even though the heating element is on, cooking your culture).

Connect the media vessel tubing to the pump tubing by twisting the two luer lock fittings tightly together. Make sure to do this using lots of 70 % ethanol.

Get an autoclaved 2 L Erlenmeyer flask or bottle. Punch a hole in the foil cover with a pencil. Remove the foil from the effluent tubing and insert the end into the flask.

Connecting the air

Make sure the air bubblers are filled with water. If necessary, top them off with milliQ water. The top of each bubbler should be held on by 1 or 2 springs (make sure at least one spring is on). If any of these have snapped, replace them.

Unwrap an autoclaved air filter and the end of the air input tubing. Snap the large end of the filter onto the luer fitting. Trace the tubing from the air pressure gauge past the air bubbler to find the end. Slip the tubing over the small end of the filter. (most bubblers have numbers on them that correspond to the position on the bank).

Open up the airflow at the gauge. You CAN blow something up by opening it too fast. When you see the little ball start to float, dial the airflow down so the bottom of the ball is just hovering above the bottom of the gauge at the 6 marking. Check the bubbler and the vessel to make sure air is flowing. If the vessel was filled with water, the overflow should start ejecting from the effluent tube. If it seems like air is escaping somewhere, make sure the top of the bubbler is secure. Other possible problems are unclipped ports, broken clips, and misaligned O-rings. The last problem can sometimes be fixed by tightening the clamp around the top of the vessel. If you cannot fix the problem, you will have to re-autoclave after fixing the problem...most likely the O-ring.

Starting the media flow

If you are using the dissolved oxygen probes, you'll need to eject all the water/media in the vessel. Use the ruler to set the height of the tube and tighten the screw when all the water has ejected.

Unclip the media line and open any clips on the tubing. Media should start flowing. If the media does not start flowing try putting the pump tubing in the pump head and tightening...then click it down a few times. If this doesn't work, remove the tubing from the pump head. Place a tube on the air filter on the carboy. Blow into the tube until media starts flowing (don't pass out doing this!). Once media has started flowing, monitor the media level in the vessel. Once it approaches the drop tube, clip the tubing.

Setting up the Pump

Unscrew the casing on the pump head. Lift up the black plastic part and thread the pump tubing through the little metal catch in the back. Pull the tubing straight out from the catch and through the opening in the black plastic part. Don't pull too hard or the tubing can pull off exposing the tube to possible contamination. Screw the pump head all the way back down.

The objective is to position the pump tubing just right so that no media flows when the pump is tightened all the way down. You also want the media to flow evenly and immediately when the pump head contacts the pump driver (that shiny rotating bar you see behind the pump heads). It might require repositioning the tubing several times to get this right. Even then, the pump may need more adjusting later.

If the pump head is squeaking or skipping, and repositioning doesn't help, try wiping the rubber grip with a damp Kim wipe. Back dust tends to accumulate as the rubber degrades and that interferes with the grip. Try switching to a different pump head if all else fails. Another trick is to loop a rubber band from the screw to the metal bars along the back of the machine. This seems to give the pumps a little extra boost. Be careful of this as you can give it too good of a boost.

Calibrating the DO Probe

Let the probe sit in media with the bubbler going and the rotors on for approximately 2 hours before calibrating. To calibrate, go to the calibration menu the same way as for the pH probe. Choose DO instead of pH from the parameter menu. The DO probe calibration only requires a high read. Scroll to the mV high read and wait until it stabilizes. When it does, hit enter. Note the mV reading when calibrating the probes. The range should be large. If it is not, there may be a problem with the probe. Once you calibrate the probe, check to make sure the probe is reading 100 in the status screen. If it is not, attempt to recalibrate. Note any problems with probes (i.e. reading over 100% DO) during the run in detail on the probe log.

Controlling pH

pH control in chemostats ensures that pH is comparable between nutrient limitations and dilution rates. It is

essential for a fair comparison of growth, physiology and various omics between conditions. Viktor Boer highly recommends pH control

to set up pH control:

Chemostat vessel:

Make sure there are two sets of pump tubing attached to the chemostat, one for supplying the medium, and one for supplying base (adding acid is usually not necessary during steady state growth, because growing yeast will acidify the medium).

Use the same type of connectors on both pump tubings.

Calibrate a pH electrode as described above.

Base reservoir:

500 ml pyrex bottles work well. Make sure there are two openings in the cap, 1 for the flow of liquid, and 1 to allow air in. For the latter, use the same connector as on the sparger (air in) on the chemostat vessel, to attach a sterile filter after autoclaving.

Attach a T-splitter for the liquid flow when desired. Again, the connectors are the same as on the medium carboy, allowing it to be attached to the pump tubing. Make sure that the tubing is long enough to approximately reach the pump head. Because in the current setup there is room for the 500 ml bottles next to the display, tubing does not need to be as long as the ones on the medium carboys.

Base:

The buffer capacity of the culture will influence how much base needs to be added to elevate the pH. Low-density cultures (50 klett) and phosphate-limited cultures generally have a low buffer capacity, so for these cultures, use 0.1 M KOH. For other cultures, use 0.2 M KOH.

For phosphate limitation, 400 ml of 0.1 M KOH is enough to keep the culture at pH 5.0 for the duration of one carboy of medium.

Make 0.1 M or 0.2 M Potassium Hydroxide and fill base reservoir with about 400 ml. Make sure that liquid cannot flow by using metal clamps. Leave the airport open, but cover all endings with aluminum foil. Autoclave 15 minutes, 121 °C.

Connecting base reservoir to chemostat

Note: when using pH control, use the base pump for base and the AF pump for the medium

After also having autoclaved the chemostat vessel, connect base reservoir tubing with pump tubing of the chemostat

Insert pump tubing in the pump head. For base, use the base pump. Remove clamps and manually press the pump head to let base flow. Fill the tubing, except for the last inch of tubing at the very end.

Programming pH control:

First, read about Programming the Chemostat, below.

Programming the controller unit is not always intuitive, and many times you have to input parameters at various locations in the menu, so check, check again, and double check while running your experiment, especially when switching profiles.

In Parameter Menu, pH, Setpoint, make sure that the setpoint is set at the appropriate pH (normally pH 5)
In Edit Profile, profiles "0" and "1" make sure that pH is "ON" and at "5"

In Edit Profile, profiles "0" and "1" Pumps, set
Acid to "Auto" and "1"
Base to "Auto" and "1"

In Pump/Flow settings, set Acid Pump to "Auto", cycle "16", set "1"

In Pump/Flow settings, set Base Pump to "Auto", cycle "16", set "1"

Note: the "set" is a parameter that you cannot actually control when the pump setting is on "Auto", however, I set it to "1" anyway, because I believe that is what it will initially start using when the pH control kicks in.

When starting your chemostat, set pH control to "On"

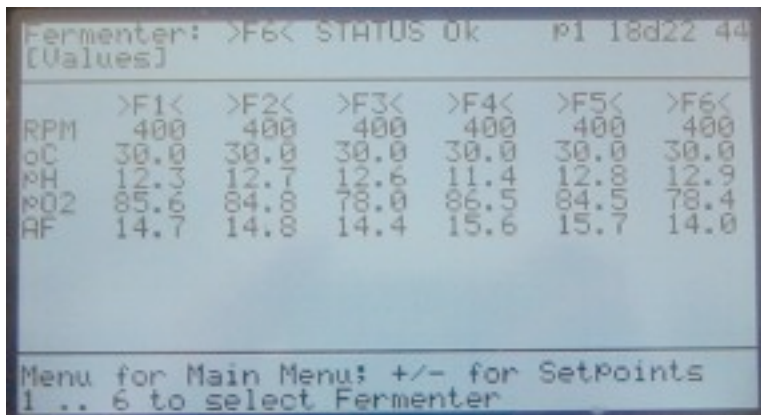
Programming the chemostat

Each vessel of the chemostat needs to be individually programmed. The chemostat interface can be counterintuitive sometimes, so be careful and double check all your settings.

Enter usually accepts a new value and Esc usually cancels it. When changing entries, you must hit enter after a numeric entry to accept the change. The +/- key changes a toggle field. For toggle entries, don't hit enter after changing them unless it's the last change you make on the screen. Esc also cancels a menu screen, leading you to the previous menu.

Profiles

For each fermenter, you need a profile or chain of linked profiles to control the fermenter parameters. Typically, you will run the chemostat in batch overnight before turning on the pumps. This involves two linked programs. First, use the esc key to get to the main menu. The esc key will now toggle between the main menu and the status screen, which shows data for all the vessels, only if the vessels are ON.



The screenshot shows a terminal window with the following text:

```
Fermenter: >F6< STATUS Ok P1 18d22 44  
[Values]  
    >F1< >F2< >F3< >F4< >F5< >F6<  
RPM  400  400  400  400  400  400  
oC   30.0  30.0  30.0  30.0  30.0  30.0  
pH   12.3  12.7  12.6  11.4  12.8  12.9  
%O2  85.6  84.8  78.0  86.5  84.5  78.4  
AF   14.7  14.8  14.4  15.6  15.7  14.0  
  
Menu for Main Menu: +/- for SetPoints  
1 .. 6 to select Fermenter
```

Figure 2. Status screen.

From the main menu, select *1 fermenter menu*. Enter the desired fermenter number at the prompt. You're now in the Fermenter menu.

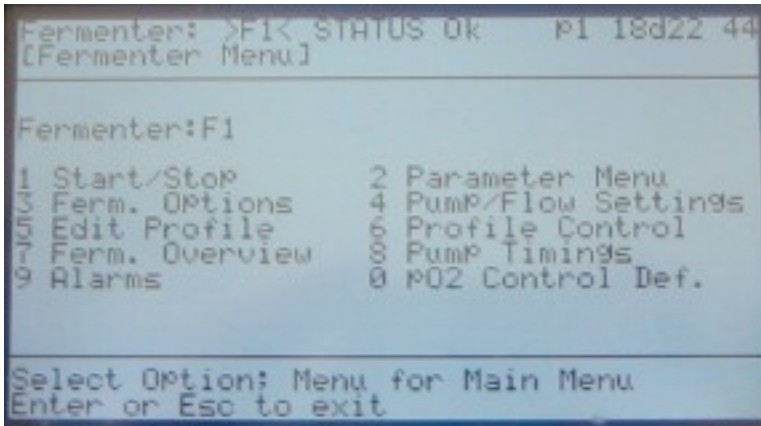


Figure 3. Fermenter menu.

Select *5 Edit Profile*. Generally, you will be editing pre-existing profiles. Input the number of the profile you want to edit and hit enter. Profile 0 is usually set up to run 24 hours in batch (i.e. no pumps) with temperature control and mixing. The standard temperature for a yeast chemostat is 30 and the mixing is 400 RPM. Make sure these settings are correct. If you need to edit a setting, use the arrows to scroll to the field and type in the new number. Hit enter to accept the change. For toggle fields, such as turning pumps on and off, you do not need to hit enter to accept the change. Once you have finished the edits, hit enter again to accept all the changes. You should be back in the fermenter menu. Hit 5 to go back to Edit Profiles and make sure your changes were accepted.

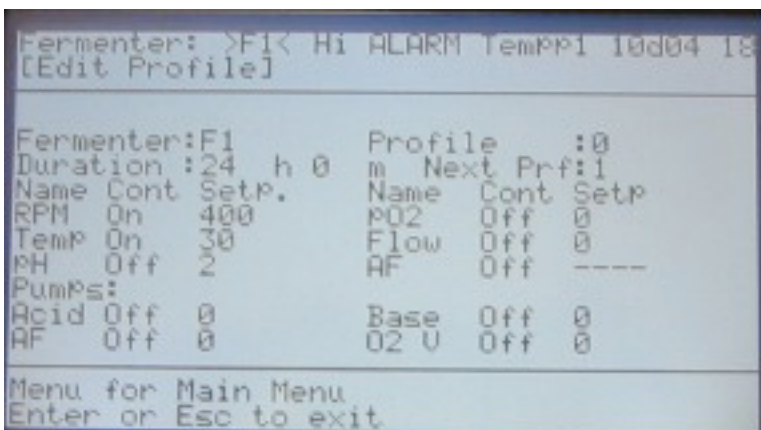


Figure 4. Edit profile 0.

Note that you can only edit one profile each time you go into the Edit Profiles screen. If you try to edit more than that, only your most recently edited profile will actually accept the changes.

You can link programs using the next profile field. The next profile can repeat indefinitely by running for any amount of time and then linking to itself as the next profile. The only difference between profile 0 and profile 1 is the addition of pump settings. Choose which pump you want to use and scroll to the on/off/auto field. Hit the arrow keys until the pump is on. Scroll to the numerical field for the pump and enter the number of counts the pumps should operate each cycle (refer to table below). You'll set the cycle counts in a different menu. Hit enter to accept the change and enter again to return to the fermenter menu. You may want to return to the edit profile menu to double check the settings.

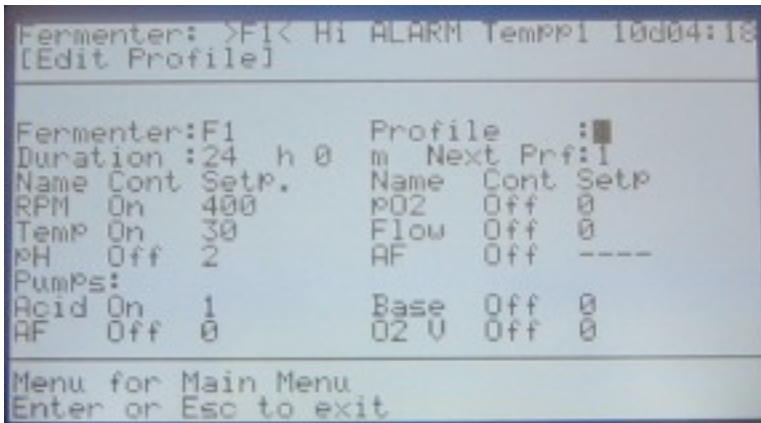


Figure 5. Edit profile 1.

To set the pump cycle, hit 4 *pump flow settings*. (see table at back of manual for common settings) You need to edit the cycle and set count. Acid and base pumps must have the same cycle count, so editing one of them updates the other automatically. The AF pump can be on a different cycle. Hit enter to accept any changes and return to the fermenter menu. The cycle counts have to match what you put in the Edit Profile menu.

Now, you need to tell the chemostat which profiles to run. To do this, go into 6 *profile control*. Set *actual profile* to be the starting profile (i.e., 0) and *last profile* the final profile in the chain or loop (i.e., 1). If you want

to run batch for more than 24 hours, either change the time of Profile 0 in Edit Profile OR have the last profile be Zero. This means that the chemostat will run in batch on profile 0 until you change it. Make sure if you do this that the next profile in the Edit Profile menu is 0.

Hitting reset profile restarts the timer for the actual profile. This feature will be used below to restart the 24 hour batch countdown once all the vessels are inoculated. If you stop and restart the chemostats, you should check the profile control window to make sure it remembered which profile to use. Sometimes it will change to the mysterious profile -1 instead of reverting to the profile it was running. If you chemostat switches to Profile -1 you have made an error in editing the profiles. Make sure the last profile in 6 is the one you intend to switch to... for example if you are in batch and switching to 1 it should say Next Profile 1.

Finally, to start the chemostat, go into *1 start/stop* from the fermenter menu. Scroll down to *start fermenter* and toggle it to on. Hit enter. The impeller should start to spin up, and the temperature will start to adjust. To monitor the progress, use the esc key to return all the way up to the status page. If you hear an unpleasant grinding noise stop the chemostat. This means the rotors are not at the appropriate height. See Sandy for how to fix this. Do not let chemostats run if they are grinding.

It is usually a good idea to stop the chemostat whenever you need to edit anything. Return to *1 fermenter menu* and *1 start/stop*. Toggle the field to on and hit enter. Check the profile control before restarting.

NOTE: All numbers should match in all fields!!

Inoculating the chemostats

Inoculate the chemostat with fresh overnight culture made from a fresh colony. Plan on 2-3 days to grow colonies streaked from a frozen glycerol stock, plus another day for the overnight culture. When possible, grow the overnight in the chemostat media you are using. Keeping the 100mL bottle from the filtering step is an easy way to do this.

Turn off the air and open a port on the chemostat. Quickly pipet your overnight into the vessel. Try to pipet straight down so cells don't get stuck on the side of the vessel.

Empty all of the effluent vessels of any extra media or water.

Recording Data

You can set up the computer to monitor and record data from the chemostat such as pH, DO, RPM, temperature etc. See manual in chemostat room for further details.

Sampling

Since you will take a Klett reading anyway, the easiest way to collect a sample is to put the end of the effluent tube in a sterile Klett tube (only has to be a sterile Klett tube if you plan to freeze down culture), resting the end near the top so air bubbling doesn't make the tube overflow. Write down the time. The Klett requires about 5-10 ml of culture for a good reading. That volume will take a few minutes to collect. While the tube is filling, pour the effluent into a graduated cylinder and write down the volume. You'll use this measurement to calculate the dilution rate later.

Record data however you like. See Appendix for a sample worksheet. Notecards are also useful.

Klett

Once you've collected about 10 ml in the klett tube, cap it. Check that the media only blank is still reading 0. Make sure your blank tube and sample tube are the same kind of tube (i.e. they both have lines or they don't have line...but do not have one tube with lines and one without). Put the tube in the Klett and turn the big knob on the

front until the little arrow lines up perfectly with the line on the meter. Record the reading.

Glycerol stock

If you are freezing aliquots of the culture, pipet 1 ml culture into 0.5 ml 50% sterile glycerol in a clearly labeled cryovial. Invert a few times to mix well and put the sample at -80C.

Sonicator

Pour ~5 ml culture into a plastic round-bottom 15 ml tube. Do NOT sonicate in glass tubes! Wear safety glasses and ear covers when you use the sonicator. Check the tip occasionally for cracks and other signs of wear. The tip needs to be replaced every once in a while. Use Program 1 on the Botstein sonicator.

Wipe the tip of the sonicator with a kimwipe saturated in ethanol. Completely immerse the narrowest part of the tip in the tube of culture. Press Start. The pulses should be evenly spaced and of equal length. The sound should not be a high-pitched squeal. You'll get to know the usual behavior of the sonicator after using it a few times. If it deviates from the usual behavior, let someone know. Wipe the tip with the kimwipe. Keep repeating until you've finished all your samples. Clean the tip thoroughly with ethanol and turn the machine off when you are finished.

Do NOT change Program 1. If you are unsure of how to use the sonicator ASK before starting.

Coulter counter

The Coulter counter interface is not the most user friendly in the world, and unguided button-pushing can completely screw up the settings. Check the manual or talk to someone if you need help. The machine will usually be in the Instrument functions screen when you start using it. Hit the *Output* button to change to the Analysis screen. Make sure the dilution setting is correct. It is a 1/1000 dilution. Check that a sample of blank diluent gives a low count on the Coulter counter. Fill a clean cup with ~20 ml

fresh IsotonII. Lower the platform and place the cup in the space. Return the platform to the top, and hit *start*. If the count is higher than about 10^6 cells/ml, clean the machine by flushing with fresh diluent and then recounting until the count is reasonable.

Once you are satisfied, vortex the sonicated sample. Add 10 μ l culture to 10 ml IsotonII in a glass tube. Avoid carrying extra beads of culture on the outside of the pipet tip. Vortex the sample well. Pour it into a cup and place it in the holder. Hit *start*. Listen for the clicks as it counts. The clicks should be evenly spaced. Look at the window with the view of the sampling aperture. It should be free of debris. The screen meter that monitors concurrent particles (CONC) should stay constant and low. If any of these conditions are not met, your count will be inaccurate, in which case you should recount the sample. If the sample itself seems contaminated by lint or whatever, make a new 1/1000 dilution in fresh diluent. If debris seems stuck in the aperture, use the *Unblock* button to try to flush it. You want the total number of particles counted to be about 10000-20000 to get an accurate estimate. You may need to make a different dilution if your sample is more or less dense than normal.

Once you are satisfied with the count, write down the particles/ml. Hit *output* to go to the next screen. Scroll the *stat* field until the mean volume is displayed. Write that down. If desired, draw the particle size distribution. When you're finished, hit the *Functions* button to return to the Instrument functions screen. Place a sample of fresh IsotonII in the holder and select Flush aperture to clean up.

Plating for viable counts

Vortex your sonicated sample again. Use it to make appropriate serial dilutions to plate for viable cell counts. Typically, I plate 100 μ l of a 10^{-4} dilution, made by 4 dilutions of 100 μ l culture into 900 μ l water or 2 dilutions of 10 μ l culture into 990 μ l water. Pay attention to your pipetting technique to ensure accurate dilutions. Pipet 100 μ l of the final dilution onto a (labeled) plate and spread evenly by your favorite method. I prefer a turntable and sterile 0.2 ml glass pipet. If your colony counts start getting above ~300/plate, you should plate less. I shoot for 100-300 colonies/plate.

Sampling for RNA

~5 ml culture is adequate to ensure appropriate yield for an RNA microarray, and it is such a small fraction of a 300 ml culture that it should not perturb the chemostat much. First, label a 15 ml Falcon tube for the filtrate and a 2 ml eppendorf tube for the filter. Using a ring stand, set up the small filter apparatus with the stopper assembly. The stopper is just 2 connector fittings jammed into the top of a Falcon tube-sized stopper. One of the fittings is connected by a piece of tubing to the filter and the other goes to the vacuum. Fit the stopper into the 15 ml Falcon tube for the filtrate. Clamp or screw down a 25 mm filter in the filter apparatus, depending on which one you're using. Hook the vacuum tubing to the other stopper port. Rinse the apparatus with milliQ water before using to remove any trace of previous samples. Even a tiny bit of, say, sulfur-limited media contains a huge amount of phosphate relative to the filtrate from a phosphate-limited culture.



Figure 6. Small filtering apparatus.

You also need a bucket of liquid nitrogen. Get another Falcon tube to collect the culture. Loosen the screw holding the drop tube. Put the end of the effluent tubing in the Falcon tube. Press the effluent tube down until it just touches the surface of the culture. You should have about 5-10 ml of culture in the tube. Turn on the vacuum and dump the culture into the filter assembly. Let it vacuum through. Remove the clamp and glass funnel. Disconnect the vacuum. The order is important to prevent cells from sticking to the glass funnel and to allow all the filtrate to get sucked into the collection tube. Without disturbing the film of cells, remove the filter with a spatula. Fold it and insert into the 2 ml locking

eppendorf tube. Close the tube and put it in the liquid nitrogen. Readjust the drop tube to the appropriate height. Rinse the filter apparatus with milliQ water and repeat for the remaining chemostats. When you've finished collecting all your samples, transfer the tubes to -80°C . Clean up after yourself!!

Media Replacement

You will need to change the media carboy every few days. Since we don't have an excess of carboys, please don't stockpile media too much in advance. Sample before you change the media.

If you want to get all the media out of the carboy, there are 2 techniques used:

If you are running chemostats off of just one carboy, you will have to use this first option. When you are down to the last bit of media, put an eppendorf tube rack under the front of the carboy to tilt it back and pool the remaining media. Adjust the metal drop tube feeding into the chemostat so that it sits at the deepest part of the pool. Check that you didn't break the siphon. You should be able to get almost all the remaining media this way.

The second technique works if you are feeding your chemostats off two media vessels connected by a Y connector. You can replace a media carboy whenever the total media remaining in both carboys will fit into only one. First, drain all the media into one carboy by placing one carboy on a lower shelf than the other. The lower carboy should start to fill up. You can prop up the top carboy as described above to drain the maximum amount of media. Clip the end of the tubing from the empty carboy. Replace the other media carboy on the higher shelf.

With either technique, watch the supply closely. You don't want to forget about it and run out of media. Once you've almost run out of media, or if you are leaving and the media will run out before you get back, replace the carboy. Clamp the end of the tubing, untwist the fitting, and twist it onto the new carboy's fitting. Unclamp the new carboy and watch to make sure the media starts flowing. Write down the time. Take a sample of the media for analysis.

Data Analysis

Once you've got some sampling data, you'll want to analyze it. At the beginning of a run, it's important to calculate the dilution rate to make sure the pumps are behaving and the settings are correct. The dilution rate is a simple relation of the effluent volume, length of time (in hours) effluent collected, and chemostat volume:

$$D = \text{effluent volume} / (\text{time} * \text{chemostat volume})$$

The dilution rate is in units of hr^{-1} . It is also sometimes called omega.

Since your chemostats will all be running at different rates, either by experimental variation or by design, generations is often a more useful metric than time for graphing things and talking about run length. The chemostat literature talks about two different types of generations: a culture generation, i.e. one volume replacement of the chemostat, and the cell generation, i.e. the doubling the cells must undergo to keep up with the dilution rate. Since some cells get diluted out before they can divide, the culture as a whole must actually double faster than the chemostat volume replacement rate. I usually calculate the cell generations elapsed since I last sampled:

$$\text{cell generations elapsed} = (\text{time} * D) / \ln 2 = 1.44 * \text{time} * D$$

You can cumulatively add up the generations for every sampling point to get a column for making scatter plots.

Ending a Run

Clean the entire chemostat well with DI water. If you have any obvious wall growth, scrub the walls with a wet paper towel. Rinse well. Place all probes, cleaned* and capped, back in the rack in appropriate numerical order. Clean up any spilled culture or media in the chemostat room. Sometimes a spill will spread under the chemostat. Try to soak it all up and wash the bench with diluted Contrad 70 or bleach. Media spills are a haven for contaminants.

Clean out all carboys well with DI water at the end of a run, or after a media change. Rinse all lines well.

*** Cleaning the DO Probes**

- After every run, the probes must be cleaned appropriately
- Remove the covering at the bottom of the probe
- Gently remove the membrane body without touching the bottom with your hand (wear gloves at all times)
- Empty out the electrode. If the electrode is dirty, rinse the membrane body with fresh electrode
- Wipe the probe with a kimwipe to remove any residue
- Fill the membrane body $\frac{1}{2}$ way with fresh electrode
- Place the probe back in the membrane body. Electrode will spill out. gently wipe the membrane body dry with a kimpwipe...IT MUST BE DRY before replacing the covering
- Replace the covering and screw it back into place
- Put the probe in the rack with a green cap covering the bottom

Problems

Problem	Explanation and Solution
Pump squeaking or skipping	Pump problems. Reposition tubing. Rubber band trick. Change pump head.
Flow rate wrong	Pump problems as above. Program wrong, check flow settings. Effluent tube not in effluent vessel (should be accompanied by spill). Kink in tubing.
Vessel fills up, won't eject effluent	Air leak. Check clips on tubing, o-ring seating, and rubber bands on bubblers
Contamination on viable count plates	Either contamination of the vessel or the water/media used to dilute the cells.

	Check vessel culture by directly sampling through one of the top ports. Replace water/media.
Washout	Dilution rate set too high. Check settings. Media missing an ingredient. Media carboy contaminated.
Mystery spill	Either water or media. If media, check for loose tubing connections or holes in the tubing. If water, check the water tubing and faucet for leaks.
Media flow stops	There are many reasons...a common one is a broken siphon. Check for air bubbles and clear them by blowing into the big air filter on the carboy. Check for holes in the tubing. Kinked tubing
Pumps all come on, screen wacky.	Corrupted memory card. See Sandy before doing this. Swap the card with another one and see if that solves the problem. (Port is on top section of the chemostat, on the left side. Turn it off before servicing.) Can also try reinitializing card by hitting escape as the machine turns on, then enter to initialize.
Cloudy filtrate during harvest	Faulty filter. Replace and refilter. Filter not centered on apparatus. Reposition and refilter.
Funny noises, strange behavior	Sharpie stuck inside the machine.

Unique problem with machine

Call Andy at ATR 1-800-827-5931

Common Pump Settings

<u>Volume</u>	<u>Dilution Rate</u>	<u>Pump Settings</u>
300mL	0.05	38/2
	0.56	16/1
	0.11	8/1
	0.16	16/3
	0.22	4/1
	0.30	16/5
200mL	0.06	20/1
	0.12	20/2
450mL	0.12	20/4

Date and time: _____ **time started** (_____ **h**)

Chemostat:	Strain 1	Strain 2	Strain 3	Strain 4	Strain 5
Effluent volume (mls)					
Klett units					
Coulter Counter ($\times 10^7$) Blank					
Mean size (fL)					
Dilution rate					
Doubling time					
Number of generations					
Microscope Hemacytometer					
Other					

