New Brunswick BIOFLO 5000 Fermentor and CEPA Centrifuge in LS3108A are shared instrumentation for the use of approved laboratories and approved users

To become an approved user/laboratory please contact:
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This protocol describes the operation of the fermentor and centrifuge for simple use in growing large batch cultures of bacteria or yeast primarily with the intent to purify recombinant proteins. This operation forgoes many of the possible controls but is adequate for the purpose.

If you want to operate the fermentor in a more sophisticated fashion, please contact Wolf-Dietrich Heyer.

Overall Notes on Operation

- The operation monitors pH but does not control pH.
- The operation monitors dissolved oxygen (DO) but does not control dissolved O_2 level.
- The fermentor can grow cultures between 10-50 L. 10 L is the minimum to cover the impellor and 60 L is the maximum. Sterilization with all a few liters to the volume.

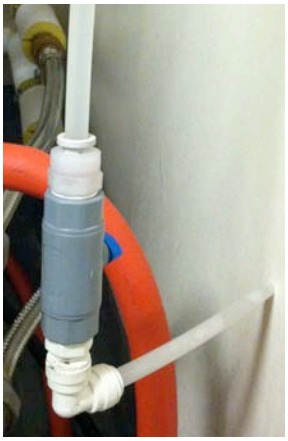
BEFORE USE: Please sign logbook in fermentor room!

Sterilization of media

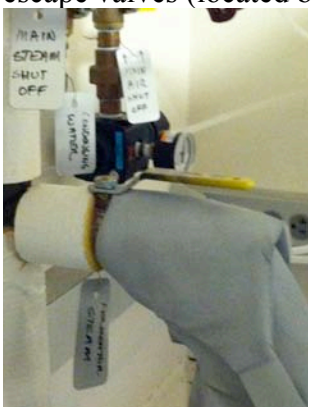
1) Before starting any of the units, turn on big red valve for water supply and gray valve that supplies water to the chiller. These are what control the temperature of the fermentor.

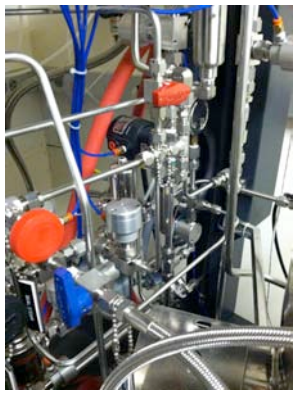
2) Turn on fermentor (switch on right bottom of machine)
3) Turn on chiller and make sure diH_2O valve is open and set to 6°C.

4) Add ingredients for media using top lid of vessel. Use diH_2O (shown below) to wash canisters. Make sure to add 2 mL Antifoam agent (located on desk in fermentor room).



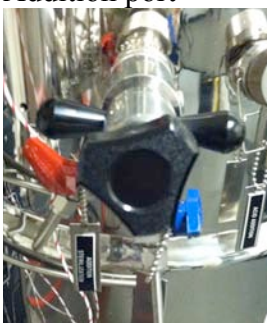
5) Add water to complete volume. Demarcations indicate where 40, 50, and 60L liquid levels should appear on the fermentor window.
6) Turn on yellow valve that supplies steam to the fermentor. Make sure red and blue air escape valves (located behind vessel) are closed.





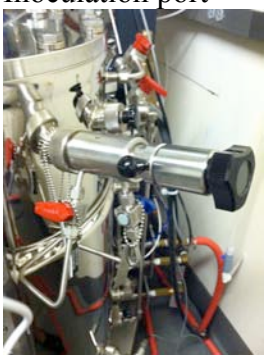
7) To start sterilization process, go to Sterilization menu on fermentor by pressing the <SCREEN> button. The temperature is pre-set to 121°C and it must be turned <ON>. In addition, the agitation should be set to 100 at P-I-D.
    a. To set any condition on fermentor controller, must use arrows to scroll to the specific condition that needs to be changed, then press <ALTER> button until the correct choice is showing then press <ENTER>.
8) Start autoclave and wait until temperature reached 121°C.
9) At 121°C, open "upper" large red valve on the top right of the fermentor for 20 min. This will sterilize the pipes through which air will travel to the top of the fermentor.

10) While this happens, sterilize all ports ONE AT A TIME for 10-20 seconds. First, open red valve associated with the port, wait five seconds, then open blue valve. After 10-20 seconds minimally (or until the valve is hot to the touch – BE CAREFUL if you touch the valve! Jie recommends 1 min) close the red valve, wait five seconds, and close blue valve.
    a. Addition port



    b. Sampling port (does not have blue valve associated with it.)

    c. Inoculation port



    d. Drain ports. There are two that are to be sterilized separately:

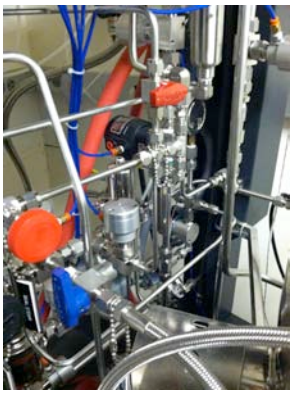
i. Drain Port: Lower red valve with silver valve located on hose
ii. Drain Ball valve: top red valve and blue valve on the lower right of fermentor.


11) After 20 minutes, close the "upper" large red valve and open the "lower" red valve for 20 min. This will sterilize the pipes through which the air travels to the bottom of the fermentor.

NOTE: The fermentor will automatically cool down after sterilization but it takes a while, so it may be better to make media the day before inoculation.

Inoculation, Growth, and Induction

1) Turn on red valve, diH2O valve, steam valve (yellow valve in the back), fermentor, chiller and open air escape valves (red and blue valves that are behind the vessel). Also open the "lower" air valve for air to go into vessel from the bottom.
    a. Different chiller temperatures for different growth temperatures:
        i. For 20°C or lower set chiller to 5°C
        ii. For 30°C set chiller to 10°C
        iii. For 37°C set chiller to 15°C



2) Set agitation to 55, temperature to 30°C and turn Air to 65 and make sure it says <ON>. The pressure at this point should be at 0.8. If it is too low or too high, can adjust with the black rotating knob behind the fermentor.
3) Calibrate DO by removing DO probe (lower right) and in the calibration menu, select <ZERO> to set the zero point and wait until the reading it zero, then type in "0.0" and press <ENTER>.
4) To set maximum DO, set agitation to 500 and see the DO reading go up. Can set this for 30 min. Alternatively, can keep agitation at 100 and just wait 30 min for DO to stabilize. Then, select <SPAN> in the DO menu and type in 100.0. This will represent 100% of

the oxygen in the water and as the culture grows, one can monitor the percentage of oxygen consumed.

5) Depressurize vessel to add inoculate by:
    a. Turn Air to <OFF> and turn off agitation
    b. Sterilize pressure port (also called addition port)
    c. Uncap the port
    d. Push slowly (be careful, first air may be hot) then turn clockwise and let all the pressure out
    e. Open the top of the vessel but DO NOT unscrew completely until you recapped the pressure valve and have re-sterilized it.
    f. Add culture, then close vessel, Put air back to <On> and agitation to <On>.
6) Take OD measurements every 4-6 hours to make sure that the sample is growing well. Can do this through the sample port. Make sure to sterilize before and after. Use big jug to collect the culture that first comes out, then use a conical tube to collect some sample for OD measurement.
7) Before induction take pre-induction sample. 15-25 mL is okay (I used 15 mL). I like to induce between 2 and 3 ODs. Add galactose similarly to how inoculate was added.

Centrifugation

1) Take post-induction sample (See above).
2) Turn on centrifuge to warm the machine up by rotating black knob. You cannot use it until the light is on under "Operating Centrifuge". This takes roughly 15 minutes.

3) While the machine is warming up, assemble rotor and centrifuge parts (see manual).
    a. Must grease all inner linings of all parts of the centrifuge. Grease is located on desk in fermentor room.
    b. First, assemble bottom of rotor. Make sure disk above the spring is centered. Will need tools kept in Rita's desk to tighten parts.




c. Then assemble the centrifuge tube. Follow the picture in the manual to place the teflon sheet in correctly. This is very important because there will be cell loss if improperly placed.

d. Place rotor carefully on the spindle. Be very careful not to knock the top of the centrifuge, and use dark gray screw cap to protect the metal bearings.

e. Put on the lid, pointing the part where the supernatant will come out as far away from the machine as possible (to help in supernatant collection). Take off plastic cap on lid and screw into storage thread on centrifuge, grease the threads.
f. Push in the head bearing spindle from the top of the cylinder, check that the teeth on the spindle alternate with notches to make sure bearings are properly aligned when securing the centrifuge tube (Viewed from the top hole which is covered by a plastic lid). Can only descend the locked part that connects the tube to the centrifuge when the light of the "operating centrifuge" is on.

g. Tighten clockwise using two spanners
h. Lo←wer large metal sleeve and tighten it

4) Sterilize the hose for 2-5 minutes by opening small red valve in lower left of the fermentor and the silver valve to the right of the blue valve. Make sure steam is going to the drain. Sterilize drain ball valve as well (upper red valve and blue valve on lower right of fermentor.
5) Switch the air-flow from the "lower" red valve to the "upper" red valve.
6) Connect the hose to the bottom of the centrifuge.
7) Don't let cells flow until you have cooled the drain port that was just sterilized. Can cool by placing ice on the metal part but be careful not to burn your hands. Can also do this step 30 min prior to centrifugation.
8) Check to make sure the the latch on the metal drain port connected to the hose is CLOSED to avoid accidental loss of cells.
9) Open yellow handle on red tubing behind the fermentor to flow water from the chiller to the centrifuge. Make sure the centrifuge unit is cooled before starting.

10) Place container for supernatant (located underneath the desk in the fermentor room) right underneath head of centrifuge and add 1 spoon of germicide (on desk in fermentor room).
11) Set up air to 1.5 then increase to 5. After the liquid level is lower than the bottom propeller, can increase to 10. DO NOT OPEN upper lid on the top of the fermentor. Keep closed!
12) When the light is on start to centrifuge the cells by pressing the green "Centrifuge On' button. 40 L takes roughly 30 minutes. When centrifuge is at full speed, open blue valve on the bottom center of the fermentor connected to the hose to let the cells out of the vessel.
13) Take samples of the supernatant every 10 minutes to verify that it is clear. When it first comes out, there is a lot of air bubbles, but when it settles it should be clear.
14) When centrifugation is complete (vessel is empty), turn off centrifuge with red "Centrifuge Off" button. It takes 10 minutes before standstill light comes on and rotor can be dismantled.
15) Reverse assembly instructions. Slide metal sleeve up. Use two spanner to untighten. Push spindle up in lock position. Use protected dark gray cap on top of rotor. Spindle can only be locked when the light of the machine is on. After that, you can shut off the machine with the black handle.

Cell Collection

1) While centrifugation is taking place, clean sink area in autoclave room with ethanol and spread bench paper (blue side up). You will also need the centrifuge cradle, a glass bowl (located about Rita's bench), a large plastic container (to collect the liquid that is still in the centrifuge tube), big metal spatula, pipetaid with 25 mL plastic pipets, and clean ziploc bags.
2) Cover supernatant container.
3) When you take out the centrifuge tube, immediately hold it upside down in the plastic container to collect the liquid that is still in the tube.
4) Unscrew tube using the tools and remove plastic sheet inside a Ziploc bag placed in the glass bowl. Scrape cells off with spatula.
5) To further clean the plastic sheet, use pipetaid to clean all cells from the plastic sheet into the plastic container with the supernatant.
6) Can directly freeze Ziploc bag of cells after weighing in liquid nitrogen. Use 1L bottles to spin down supernatant and collect those in 50 mL conical tubes. Weigh and freeze cells in liquid nitrogen then freeze long term in -80°C.

Clean up

IMMEDIATELY:

1) MUST disassemble all centrifuge parts and clean (make sure all grease is off, there should be no black residue on any of the parts) and place to dry in cart located in fermentor room.
2) After 30 minutes in germicide, the supernatant can be drained into floor drain in fermentor room (LS3108A). Clean container by washing with diH_2O and draint content to leave container dry and empty.
3) Wash the inside of the fermentor the day of to make sure no cells are crusted to the inside of the tank.

WITHIN THE NEXT 24 HOURS:

4) MUST sterilize water in the fermentor either day of or day after to get the fermentor ready to the next user.
5) Clean and decontaminate floor, cart and sink with bleach or Vikron, flush with water and clean floor with mop.
6) Switch off all instruments (centrifuge, chiller, fermentor) and close all handles.
7) Log out in logbook.

Biological Containment

- Make sure your laboratory has an active Biological Use Authorization (BUA), which is required for large cultures even exempt of organisms.
- A copy of the Heyer laboratory BUA is found on the shelf in the fermentor room (LS3108A). For more information contact UCDavis EH&S Biosafety Officer at 2-1493.
- All contaminations, spills, and effluent from the centrifuge must be deactivated with either Chlorox bleach (final concentration is 10%) or Vikron (1% weight:volume) for 1 hour.

Ingredients

For 40 mL Basic Media

268 g Yeast Nitrogen Base w/o Amino Acids
34.8 g Drop-out mix
1200 mL Glycerol (3%)
800 mL Lactate (2%)

For 1 L 20% galactose (will need 4 L for 40 L of cells and autoclave beforehand)

200 g D-galactose