Microbiology Lab Cookbook

Total Organic Carbon Analysis

Powering-up the unit:

1. Press power switch into the "on" position. The power switch can be found at the lower right side on the back of the instrument (not the monitor).

2. Be sure that the screen comes up. If it doesn't, then the dimmer knobs may need to be adjusted.

3. When the "Choice" prompt comes up, hit "1" to launch TOC software.

4. Open the Oxygen tank after a small rectangle has appeared in the center of the screen containing the message "Low gas pressure. Hit escape to refresh." Hit escape first, then open the cylinder all the way.

5. To allow the furnace to heat up, adjust the external regulator pressure on the Oxygen tank to 50-60psi. Press F3. Look under "Status" at the furnace temp. Make sure that it is increasing. Allow about 20 minutes for it to reach 680 degrees.

6. Once the furnace has reached 680 degrees, a "System Ready" statement will appear in the Unit Status box in the upper right hand corner of the screen.

7. Before running anything, make sure that the water level in the humidifier is above the red line. If it is not, then unscrew the cap, discard the old water and add fresh milliQ water until it is a little above the red line.

Cycling Clean Water Through the System:

Fresh milliQ water should be cycled through the system before running any standards or samples. This is done to remove contaminants from the analyzer.

To do so:

8. Obtain fresh milliQ water using the 200ml volumetric flask which is next to the instrument. Be sure to soak in acid and rinse 3x with QH2O if it hasn't been used in a while.

9. Press F5 to get to the Configuration Screen. Make sure that sample intro is set on "sipper".

10. Use the arrow keys to highlight Mode. Use the Pg. Up/Pg. Down key to select TC. Hit enter.

11. Press F4 to bring up the Sequence Screen. From this screen run sequnces can be created and standard type can be programmed. Hit 3 for sample, 1 for quantity, 20 for reps, then enter to get to the next line. If the instrument was run the day before, then enter only 10 for reps.

*If you make a mistake while entering sequence info, then hit C to clear the screen or E for edit.

12. Enter a calibration sequence on the same table. Hit 2 for Standard, 1 for quantity, 4 for reps, then enter. Repeat this step three more times(one line for each of the four standards: blank, 1mg/L, 5mg/L, 10mg/L).

13. Enter a sample sequnce under the calibration sequence. Hit 3 for sample, 1 for quantity, 4 for reps, then enter. Repeat this step for as many samples as you will be running that day.

14. Place the sipper tube into the water-filled volumetric flask. Be sure that the tip of the tube reaches all the way to the very bottom of the flask and secure it with tape. This is to ensure that no air is sucked up during cycling.

15. Press F1 to begin cycling. This will take about an hour. Since this does not require close supervision, now would be a good time to defrost standards. Pull out a blank, 1mg, 5 mg, and 10 mg standard from Freezer # 3. Set these in a water bath on the sink counter.

16. Once the water cycling sequence has ended, a “Warning: Change sample and press start to continue.” message will appear in the center of the screen. This means that the instrument is ready to run standards.

Running Standards:

17. Wear gloves.

18. Make sure that the standards are fully defrosted.

19. Go back to the sequence screen. Look at “Standard type” to make sure that it is set for TC mode. If it isn’t, then hit M for modify standard. Use the Pg up/Pg down keys to select TC. Hit enter, then escape.

20. Place all defrosted standards under the hood. Pipette 25ul of Ultrex HCl into each vial. Recap and place near the instument.

21. Open the Nitrogen cylinder all the way.

22. Insert a pre-combusted, borosilicate glass capillary into the tubing attached to the N_2 regulator.

23. Starting with the blank, remove the cap and place the capillary into the vial. Adjust the regulator so that a gentle stream of bubbles flows through the standard. **Warning:** If you allow too much gas to flow through the sample, then you risk blowing a significant volume of it out of the vial/flask(and possibly into your eyes!). Sparge in this manner for 1 minute.

24. After sparging is completed, discard the used capillary. Place the end of the sipper tube all the way down into the bottom of the vial, secure with tape, and press F1 to begin the sequence.

25. Record the standards in the log book as they are run. Standards are to be run in increasing order of concentration ie. blank, 1mg/L, 5mg/L, 10mg/L.

26. After each standard is completed, a message will appear in the center of the screen to change the sample. Follow the same directions as in step 9.

27. Once all standards have been run, you are ready to begin analyzing samples.

Running Samples:

28. Defrost about three or four samples at a time in the water bath.

29. Once defrosted, dilute the samples 1:10 in a clean, acid washed, 3X QH_2O rinsed volumetric flask. (Pipette 1ml sample into flask, followed by 9mls fresh QH_2O...The meniscus of the solution should be at the white line on the neck of the flask.)

30. Pipette 25ul Ultrex HCl into each flask. Place yellow cap over opening.

31. Sparge the sample you are going to run with a gentle stream of N_2 gas for 1 minute.

32. Place sipper tube into the flask until it is touching the bottom. Secure with tape. Press F1 to start.

33. Repeat steps 13-16 for each sample.

34. Note: Make sure that the variability between injections is not large.

Powering-down the Unit:

35. After the last sample is completed, press F2 twice to officially end all runs.

36. Turn off the power switch.

37. Set the timer for 10 minutes. This is to allow oxygen to flow through the instrument before it is completely shut down. Cutting off the flow of oxygen before 10 minutes may damage the instrument.

38. While waiting for the 10 minutes to end, close the N_2 cylinder.

39. After the timer goes off, close the Oxygen cylinder.

40. The instrument is completely powered down. Replace the tip of the sipper tube into the rinse carboy attached to the right side of the instrument.

Maintenance of the Instrument:

***** Ch. 5 of the instrument manual answers all maintenance questions(pgs. 37-52).

Commands:

F1 Start

F2 Hold a run(when pressed once). Stop a run(when pressed twice).

F3 Run Screen

-shows peaks

-attenuation changes peak size(up & down arrows)

-chart speed can be changed with left & right arrow keys

F4 Sequence Screen

F5 Configuration Screen

-enter parameters on this screen

F6 Calibration Screen

F7 Diagnostic Screen

F8 Error Screen

-see pg. 57 in manual

Making Standards:

Note: All glassware required for this procedure must first be soaked in 10% HCl, rinsed 3X with QH_2O, then allowed to dry completely before use.

1. Obtain a 1000ml flask.

2. Carefully weigh out 0.08503 grams of Carbon Std. Place into the flask and dilute with QH_2O. This is the 40mg/L standard stock soln.

3. To make the 20mg/L std: Transfer 100mls of 40mg/L std into a 200ml volumetric flask. Dilute with 100mls of QH_2O.

4. To make the 10mg/L std: Transfer 50mls of 40mg/L std into a 200ml volumetric flask. Dilute with QH_2O.

5. To make the 5mg/L std: Transfer 50mls of 20mg/L std to a 200ml volumetric flask. Dilute with QH_2O.

6. To make the 1mg/L std: Transfer 20mls of 10mg/L std to a 200ml volumetric flask. Dilute with QH_2O.

Note: It is important that all flasks be labeled so that the standards are not mixed up!

7. Pipette the standards into pre-combusted scintillation vials which are labeled respective to the concentration of the standard they will contain.

8. Use the same source of QH_2O to make blank standards.

9. Place all scintillation vials containing standard into the box labeled “Standards” in Freezer # 2.